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(54) Title: RECOMBINANT CONSTRUCTS ENCODING T CELL RECEPTORS SPECIFIC FOR HUMAN HLA-RESTRICTED TUMOR ANTIGENS			
(57) Abstract			
<p>Methods are described to obtain nucleic acid molecules that encode T cell receptors and their derivatives that are human HLA-restricted and which are specific for tumor-associated antigens found in human tumors. These nucleic acids are useful in preparing recombinant cells for diagnosis and therapy of human tumors.</p>			
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RECOMBINANT CONSTRUCTS ENCODING T CELL RECEPTORS SPECIFIC  
FOR HUMAN HLA-RESTRICTED TUMOR ANTIGENS

5    Technical Field

The invention is directed to recombinant T cell receptors and modified forms thereof that are useful in identifying displayed tumor antigens and in antitumor therapy.

Background Art

10           Cytotoxic T lymphocytes (CTLs) form an essential part of an immune response to infectious agents and to malignancies. Thus, CTLs which are directed to established tumors may be effective in destroying these targets. Greenberg, P.D. *Adv Immunol* (1991) 49:281-355. CTL may also be used to identify tumor-specific antigens such as MAGE, GP100, tyrosinase, and MART, as well as broadly expressed tumor-associated  
15   antigens such as P53 (Yanuck, M. *et al. Cancer Res* (1993) 53:3257-3261); Houviers, J.G.A. *et al. Eur J Immunol* (1993) 23:2072-2077; Her-2/neu (Peoples, G.E. *et al. Proc Natl Acad Sci USA* (1995) 92:432-436; Fisk, B. *et al. J Exp Med* (1995) 181:2109-2177; as well as the tumor antigen Ras (Skipper, J. *et al. J Exp Med* (1993) 177:1493-1498).

20           It has been typical that such tumor-specific CTLs have been obtained from tumor infiltrating lymphocytes (TILs). However, this is subject to a number of disadvantages due to the complexity of the system and the endogenous mechanisms to counteract the effect of these CTLs. Importantly, the most effective CTLs may have been eliminated (Schwartz, R.H. *Cell* (1989) 57:1073-1081); the target tumors may  
25   have become resistant (Browning, M.J. *et al. Curr Opin Immunol* (1992) 4:613-618); or the T cells may lose functional activity by down-regulating expression of the  $\zeta$  chain of the CD3 complex or the p<sup>56</sup> LCK molecules (Mizoguchi, H. *et al. Science* (1992) 258:1795-1798).

          In order to overcome these disadvantages, the present applicants have used  
30   transgenic mice as a source of CTLs that contain the desired nucleotide sequences

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encoding TCRs specific for tumor-associated antigens restricted by human HLAs. Both humans and HLA-A2 transgenic mice select the same A2-restricted antigenic epitopes from influenza (Vitiello, A. *et al. J Exp Med* (1991) 173:1007-1015). Also, the present applicants have shown that HLA-A2 transgenic mice can produce p53-specific, A2 restricted CTLs when immunized with certain p53 derived peptides.

5 Theobald, M. *et al. Proc Natl Acad Sci USA* (1995) 92:11993-11997.

Of course, if murine-derived TCRs are to be used in a human context, humanization of such TCRs would be advantageous. In order to avoid competition for dimerization with endogenous V $\alpha$ /C $\alpha$  or V $\beta$ /C $\beta$  TCR, it may be advantageous to

10 prepare chimeric TCRs using the  $\zeta$  region of the CD3 receptor as the transmembrane and cytoplasmic domain. Such constructs could be prepared in either dimeric or single-chain form. Competition by V $\alpha$ /C $\alpha$  or V $\beta$ /C $\beta$  for each other or for the availability of CD3 chains has already been shown by Gorochoff, *International J Cancer* (1992) 8:53-57 and by Wegener, A.M.K. *et al. Cell* (1992) 68:83. Chimeric

15 V $\alpha$ / $\zeta$  + V $\beta$ / $\zeta$  chimeras were described by Engel, I. *et al. Science* (1992) 256:1318 who also showed that such chimeras could be activated by exposure to the appropriate antigen-MHC complex. In addition, Irving, B.A. *et al. Cell* (1991) 64:891 reported that chimeric molecules composed of the CD8/ $\zeta$  or CD16/ $\zeta$  and expressed in T cells had the capacity to transduce activation signals for IL-2 production and mediated

20 specific cell lysis in a manner indistinguishable from those generated by the TCR/CD3 complex. In addition, Chung, S. *et al. Proc Natl Acad Sci USA* (1994) 91:12654-12658 constructed a single-chain TCR (scTCR) using the  $\zeta$ -chain of CD3 and expressed it in T cells, thus conferring the T cells with the relevant specificity. These T cells further produce IL-2 on activation with the specific antigen. The present

25 applicants have further confirmed this approach using clone 4 TCR as a model system.

However, there remains a need for a convenient source of nucleic acids encoding TCR molecules and their modified forms which are human HLA restricted and specific for common tumor-associated antigens. The present invention supplies this need.

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**Disclosure of the Invention**

The invention provides materials that are useful in tumor diagnosis and therapy by permitting altered T lymphocytes to recognize and destroy unwanted tumor tissue.

T cell receptor-encoding nucleic acid molecules can be obtained by immunizing  
5 transgenic mice which produce human HLA with tumor-associated antigens and recovering the nucleic acids encoding the T cell receptors from the cytotoxic T lymphocytes (CTL).

Thus, in one aspect, the invention relates to a method to prepare an isolated nucleic acid molecule comprising a nucleotide sequence encoding at least one of the  
10 variable regions of the  $\alpha$  and  $\beta$  chains of a non-human TCR which TCR is human HLA-restricted and specific for a tumor-associated antigen, which method comprises cloning or amplifying a nucleic acid molecule containing said encoding nucleotide sequence from the CTL prepared by a method which comprises immunizing a transgenic non-human vertebrate which is modified so as to express at least one human  
15 HLA antigen with said tumor-associated antigen (TAA) so as to effect the production in said mouse of cytotoxic T lymphocytes which display human HLA-restricted TCR specific for said TAA and which contain nucleic acid molecules comprising nucleotide sequences encoding the  $\alpha$  and  $\beta$  chain of said TCR and recovering the CTL.

In other aspects, the invention relates to nucleic acid molecules obtained by the  
20 foregoing method and to constructs employing their variable regions, to cells displaying TCRs or derivatives encoded by said nucleic acids or their modified forms, and use of these materials in diagnosis and therapy of human tumors.

**Brief Description of the Drawings**

25 Figure 1 shows the structure of several derivatives of effective T cell receptors wherein the  $\zeta$  region is substituted as a chimeric transmembrane and cytoplasmic region.

Figure 2 shows, in more detail, the construction of the nucleotide sequence encoding such derivatives.

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Figure 3 shows the complete nucleotide sequence and deduced amino acid sequence of a single chain TCR derivative which contains variable  $\alpha$  and  $\beta$  specific for HA linked through a short peptide linker and then fused through a CD8 hinge to the  $\zeta$  chain.

5        Figure 4 shows the ability of cells transfected with various modified TCR forms specific for HA to produce IL2 in response to stimulation with HA.

Figure 5 shows the ability of CTL's generated in mice in response to Her 2/neu-peptides H3 and H7 to mice H7 or H3 bearing targets. CTLs from both A2.1xK<sup>b</sup>xCD8

10       and from A2.1 transgenic mice were comparable in result.

Figure 6 shows the sequence of various primers useful in cloning or amplifying the nucleotide sequences in coding during variable regions of  $\alpha$  and  $\beta$  TCR chains.

Figures 7A and 7B show the nucleotide sequence and deduced amino acid sequence of the variable regions of the  $\alpha$  and  $\beta$  chains of H7-specific TCR respectively.

15       Figure 8 shows a diagram of an expression vector suitable for producing the modified TCRs of the invention.

Figure 9 shows the ability of H7 specific modified TCR forms transfected in the 27J cells to effect IL2 production in said cells in response to the H7 peptide when the H7 peptide is presented in the presence of JA2 cells.

20       Figure 10 shows the ability of the various modified H7 specific TCR constructs to stimulate IL2 production in 27J cells in response to tissues bearing Her2/neu-peptides.

#### Modes of Carrying Out the Invention

25       The invention provides a convenient source for desirable recombinant materials that are useful in therapeutic and diagnostic procedures related to human tumors. Specifically, the materials of the invention provide a means whereby enhanced populations of cells that display appropriate TCRs for identifying and destroying tumor tissue may be obtained, as well as providing cells that are useful in evaluating the

30       tumor-associated antigen that could usefully be targeted.

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Briefly, the recombinant materials are obtained from CTL produced by immunizing nonhuman subjects with tumor-associated antigens associated with human tumors, where the nonhuman subject has been modified so as to be capable of expressing a human HLA. Thus, the relevant TCRs are not only specific for the human tumor-associated antigen, but also restricted by a human HLA. While murine subjects are clearly the most convenient at the present time, further developments in the construction of transgenic animals may permit alternative nonhuman subjects to be used equally conveniently in the near future. Such additional nonhuman subjects may include rats, avian subjects, larger mammals, or any appropriate vertebrate system that can be manipulated to provide it with human HLA and which can mount an immune response to provide CTLs with the appropriate T cell receptors.

Further, while the human HLA illustrated herein is A2, there is no theoretical reason why other HLA domains such as A1, A3, and B7 could not be used as well. Because transgenic mice are readily available which produce this antigen, the use of a A2 as the restrictive antigen is simply a matter of convenience. In addition, if murine subjects are used, and the MHC region is entirely human, it is preferred to use mice transgenic so as to express human CD8 as well as human Class MHC antigen. This is due to the inability of murine CD8 to interact effectively with human A2.1. Thus, expression of human CD8 on the murine cells facilitates lysis of target antigen presenting cells. On the other hand, for mice transgenic for MHC human/mouse chimeras, such as A2K<sup>b</sup> mice also exemplified below, the presence of human CD8 is not necessary

The recombinant materials relevant to the invention include those associated with the TCR produced by the nonhuman subject *per se*, and also derivatives of this TCR which retain their HLA restriction and specificity characteristics. Such derivatives contain the variable regions of the  $\alpha$  and  $\beta$  chains either as dimers or in single chain form and are more advantageous than the nonhuman TCR *per se* for a number of reasons. First, if the desired TCR can be "humanized," less unwanted side-reactions can be expected. Second, economies of production can be effected if shorter peptides can be substituted for the TCR *per se*. Third, if the TCR is produced as a

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single chain, rather than in its customary dimeric form, economies of production and ease of association of the relevant variable units are achieved. In all cases, substituting a derivative for one or both of the  $\alpha$  and  $\beta$  chains or a single-chain form containing variable regions of both  $\alpha$  and  $\beta$  precludes the formation of hybrid TCRs wherein for example the desired TCR  $\alpha$  chain is coupled with an endogenous TCR  $\beta$ . Thus, the recovery of cells which produce the desired derivative is greater.

Figures 1 and 2 describe some typical derivatives of TCRs useful in the invention. As shown in Figure 1, a dimeric form may be constructed wherein the variable regions of both  $\alpha$  and  $\beta$  chains are directly coupled to the  $\zeta$  regions of various CD receptors such as CD3, CD8 and CD16. These  $\zeta$  regions substitute for the transmembrane and cytoplasmic regions normally associated with the TCR. In these examples, the constant region, as it is unnecessary, is eliminated in any case.

Further, in Figure 1, an alternative construction includes a CD8 hinge region between the variable region and the transmembrane portion of the  $\zeta$  chain. This spacer may assist in appropriate folding of the receptor. Similarly, in Figure 1, construction of a single chain TCR wherein the variable regions of the  $\alpha$  and  $\beta$  chains are fused through a linker and then fused to the  $\zeta$  region is shown with and without the CD8 hinge.

Figure 2 shows a pattern for construction of the relevant plasmids containing the nucleotide sequences encoding the derivatives shown in Figure 1. As shown hereinbelow, a model system wherein clone 4 TCR directed against hemagglutinin antigen (HA) was used to supply the variable region verified the operability of these approaches.

It is important to recognize that the critical feature of the nucleic acid encoding the TCR derivative is the presence of the variable regions from the  $\alpha$  and  $\beta$  chains, and that additional sequence, perhaps for added stability, including some or all of the constant region may be present. In addition, alternative transmembrane and signalling regions other than the  $\zeta$  regions exemplified above may be substituted. Thus, the recombinant materials encoding the TAA-specific, human MHC restricted TCR derivatives of the invention need only include the variable  $\alpha$  and  $\beta$  regions of the



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relevant TCR along with some additional transmembrane and signalling sequence and may further include additional non-interfering amino acid sequence.

The desired CTLs will be specific for TAAs associated with human cancers.

Typical among these is Her-2/neu since this proto-oncogene is overexpressed in many human cancers and associated with aggressive disease and malignant transformation (Press, M.S. *et al. Cancer Res* (1994) 54:5675-5682; Slamon, D. *et al. Science* (1987) 235:177-182). Other suitable tumor-associated antigens include Ras, p53, tyranase, MART, Gp100, MAGE, BAGE and MUC-1. Any desired antigen which is associated with human tumors can readily be used.

The availability of nucleic acid molecules encoding the desired TCR permits of both diagnostic and therapeutic uses. Cells displaying the TCR at their surfaces can be used as diagnostic for the TAA that is actually expressed by the tumor. In order to conduct such assays, the tumor or a portion thereof or cells derived therefrom are exposed to cells transfected to contain an expression system for the TCR or derivative and the ability of the recombinant CTLs to lyse the tumor cells is assessed. The procedure described in Theobald, M., *et al.* (1995) *supra*, may, for example, be used. In addition, an expression for the appropriate TCR may be used therapeutically by transducing such an expression system into the peripheral blood lymphocytes (PBL) CD8<sup>+</sup> T cells from a tumor-bearing host via, for example, retroviral-mediated gene transfer. Such transfer techniques are known in the art. See, for example, Kasid, A. *et al. Proc Natl Acad Sci USA* (1990) 87:473, Rosenberg, S.A. *et al. New England Journal of Medicine* (1990) 323:570. The altered CD8<sup>+</sup> cells then provide a passive form of immunotherapy. Of course, humanized forms of the TCR as the appropriate derivatives are most helpful in this application.

The following examples are intended to illustrate but not to limit the invention.

#### Preparation A

##### Model System for TCR Derivatives

Clone 4 TCR (reference) is specific for the hemagglutinin antigen (HA). As the nucleotide sequences encoding the  $\alpha$  and  $\beta$  chains of this TCR are available, constructs

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were made to mimic the intended derivatives of the TAA-specific, HLA-restricted TCR of the invention.

Briefly, four types of chimeric molecules were constructed: two are the dimers obtained as  $\alpha/\zeta$  + the  $\beta/\zeta$  and two are single-chain TCR/ $\zeta$  chimeric molecules analogous to those shown in Figure 1 herein. The complete nucleotide sequence encoding the single chain form with the CD8 hinge is shown in Figures 3A-3B. These four constructs were transfected into the T cell hybridoma MD.45-27 and the transformants were grown under neomycin selection and screened for IL-2 secretion upon stimulation with either spleen cells from Balb/c or P815(H-2<sup>d</sup>) cells pulsed with the HA-specific peptide or RENCA tumor cell line transfected with the HA gene. The results showing the levels of IL-2 produced are shown in Figure 4. As shown, none of the transfectants showed appreciable production of IL-2 in the absence of HA. Only the transfectants containing the clone 4 derivatives showed stimulation of IL-2 production when HA was present. Both single-chain forms, with and without the CD8 hinge and both dimeric forms, both with and without the CD8 hinge showed appreciable stimulation of IL-2 production when treated either with Balb/c spleen cells plus HA peptide, P815 cells plus HA peptide, or RENCA cells expressing HA at their surfaces.

20

### Example 1

#### Selection of Her-2/neu Immunogenic Peptides

Eighteen peptides were synthesized based on the sequence of the human Her-2/neu protein wherein each sequence contained the anchor motif for HLA A2.1, that is, L, I, M, V, A, T at position 2 and position 8/9/10 (Rupert, J. *et al. Cell* (1993) 74:929-937). The binding efficiency of these peptides to A2 was determined using a competition assay as described by Morrison, J. *et al. Eur J Immunol* (1992) 22:903-907. Briefly, each test peptide (10  $\mu$ g) was incubated with radiolabeled target cells (T2-A2.1/K<sup>b</sup>, 10<sup>6</sup> target cells labeled with 150  $\mu$ g <sup>51</sup>Cr at 37° for 1.5 hours) in the presence of an influenza virus matrix protein (0.1  $\mu$ g). The ability of these peptides to inhibit the binding of the influenza matrix protein peptide M1 (58-66) to A2.1 was

30

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measured by inhibition of lysis by an M1 (58-66) specific, A2.1 restricted CTL clone.

As shown in Table 1, many of the tested peptides were able to inhibit binding of the M1 peptide.

Table 1. Her-2/neu peptides used for immunization				
PEPTIDE	SEQUENCE #	SEQUENCE	IMMUNOGENICITY	% INHIBITION
H3	369-377	KIFGSLAFL	+	38
H6	444-453	TLQGLGISWL	-	56
H7	773-782	VMAGVGSPYV	+	55
H8	546-555	VLQGLPREYV	-	43
H12	48-56	HLYQGOQW	-	15
H13	689-697	RLLQETELV	-	56
H14	747-755	KIPVAIKVL	-	35
H15	789-797	CLTSTVQLV	-	33
H16	799-807	QLMPYGCLL	-	50
H17	851-859	VLVKSPNHV	-	12
H18	871-879	DIDETEHYA	-	37
H19	933-941	DLLEKGERL	-	36
H20	971-979	ELVSEFSRM	-	5
H21	971-980	ELVSEFSRMA	-	25
H22	972-980	LVSEFSRMA	-	14
H23	1016-1024	DLVDAEEYL	-	35
H24	1172-1180	TLSPGKNGV	-	57
HIV-9K	POL	KLVGKLNWA	+	80

5 The peptides were then tested for their ability to elicit an immune response *in vivo*. The peptides were administered either to A2.1/K<sup>b</sup>xCD8 or A2.1 transgenic mice and primary cultures of CTLs were generated. Mice were immunized with a mixture of 100µg of the Her-2/neu peptide with 120µg 'helper' peptide (the helper peptide is a

10 I-A<sup>b</sup> restricted peptide derived from Hepatitis B virus core protein comprising amino acid residues 128 to 140, that induces a strong CD4 helper response ) in 100µl Incomplete Freund's adjuvant. A2.1/K<sup>b</sup>xCD8 lipopolysaccharide (LPS)-blasts were prepared as stimulators for *in vitro* restimulation of spleen cells from immunized mice. These were prepared by incubating splenocytes in complete RPMI containing 25 µg/ml

15 LPS and 7 µg/ml dextran sulfate at 1.5x10<sup>6</sup> cells/ml in a total volume of 30 ml for 3

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days. Murine spleen cells, collected 10 days after immunization, were restimulated *in vitro* with the irradiated (3000rads) blasts which had bound Her-2/neu specific peptides. Six days following *in vitro* restimulation, the CTL populations were assayed for lytic activity against T2-A2.1/K<sup>b</sup> target cells preincubated with the peptide used for stimulation (15μM). The resultant Her-2/neu peptide-specific CTL populations were maintained *in vitro* by weekly restimulation. CTL populations were restimulated in 2ml cultures by incubating with 0.1-0.2 x 10<sup>6</sup> irradiated Jurkat-A2.1 cells (20,000 rad) preincubated with Her-2/neu peptide (15μM) and 5x10<sup>5</sup> irradiated C57BL/6 spleen cells (3000 rad) as fillers in complete RPMI media containing 2% (v/v) supernatant from concanavalin A stimulated spleen cells (TCGF).

The cultured cells were assayed for cytotoxicity against T2A2.1/K<sup>b</sup> target cells pulsed with the priming peptide. In the cytotoxicity assay, 10<sup>6</sup> target cells were incubated at 37°C with 150 μCi of sodium <sup>51</sup>Cr chromate for 90 minutes, in the presence or absence of specific peptide. Cells were washed three times and resuspended in 5% RPMI. For the assay, 10<sup>4</sup> <sup>51</sup>Cr-labeled target cells were incubated with different concentrations of effector cells in a final volume of 200μl in U-bottomed 96 well plates. Supernatants were removed after 4-7 hrs. at 37°C, and the percent specific lysis was determined by the formula: percent specific lysis = 100 x (experimental release-spontaneous release)/(maximum release-spontaneous release). As shown in Table 1, only the H3 and H7 peptides were able to stimulate a CTL response. (The HIV-9K peptide, known to be immunogenic, was used as a control.)

CTL populations that were specific for H3 and H7 were established from either murine strain and maintained *in vitro* by weekly restimulation. The results of testing these established cell cultures for their ability to lyse T2-labeled targets at a ratio of 1:1 in a four-hour assay in the presence of peptide H3 or H7 are shown in Figure 5. As shown, the CTLs from either murine subject were comparably effective at comparable peptide concentrations.

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Example 2Lysis of Human Tumors by H3- and H7-Specific CTL

Various tumor cell lines were characterized by FACS analysis for surface expression of A2 and Her-2/neu peptides. These tumor cells and other control tumors were preincubated or not for 24 hours in media supplemented with 20 ng/ml  $\gamma$ -IFN and 3 ng/ml TNF- $\alpha$ , as such pretreatment increases expression of MHC-1 and adhesion molecules thus enhancing their sensitivity to lysis (Fady, C. *et al. Cancer Immunother* (1993) 37:329-336; Fisk, B. *et al. Lympho and Cytokine Res* (1994) 13:125-131). In the assay, the tumor cells were mixed with the H3- or H7-specific CTL for 6 hours and lysis was measured. HIV-9K-specific CTL were used as a control. The results are shown in Table 2.

Table 2. Killing of tumor expressing Her-2/neu									
TUMOR	TYPE	A2	Her-2	H7	H7 + CYT	H3	H3 + CYT	HIV-9K	HIV-9K + CYT
MDA.MB231	BREAST	+	+	26	89	34	85	3	14
MCF-7	BREAST	+	+	7	40	7	54	3	7
BT549	BREAST	+	+	2	36	2	40	2	15
SAOS.175	OSTEOSARCOMA	+	+	27	35	27	33	18	11
U2-OS	OSTEOSARCOMA	+	+	30	62	32	91	18	24
SW480	COLON	+	+	2	17	6	50	1	4
OVCAR-5	OVARIAN	+	+	13	23	25	29	10	12
T98G	GLIOBLASTOMA	+	+	29	93	20	99	9	13
MALME-3M	MELANOMA	+	+	4	14	28	57	2	1
SKMEL-5	MELANOMA	+	+	16	40	6	38	5	4
NCI.H1355	LUNG	+	+	13	62	11	38	7	25
Hep-G2	HEPATOMA	+	+	4	29	4	20	1	8
CASKI	CERVIX	+	+	9	20	13	30	8	11
U87G	GLIOBLASTOMA	+	-	1	1	2	1	5	1
ST486	LYMPHOMA	+	-	5	8	1	1	1	1
LG-2	EBV-TRANS.	+	-	1	3	2	4	1	1
SV80	FIBROBLAST	+	-	2	2	4	8	2	2
JY	LYMPHOMA	+	-	4	2	2	1	2	1
MDA.MB435	BREAST	-	+	1	1	3	2	4	3

As shown, the CTLs were able to lyse effectively only those tumors expressing both A2 and Her-2 peptides. Further, repeating the experiment in the presence of an anti-A2 antibody significantly decreased lysis, and H3 and H7 could be extracted from the tumors using standard techniques.

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In a manner similar to that set forth above with respect to H3 and H7, A2-restricted CTLs specific for p53 have been generated. Theobald, M. *et al.* (1995) (*supra*).

### Example 3

#### 5                    Recovery of Genes Encoding Her-2/neu and p53 TCRs

The genes encoding the relevant  $\alpha$  and  $\beta$  chains of the TCR specific for H3, H7, and p53 are cloned according to the method of Zisman, B. *et al. Eur J Immunol* (1994) 24:2497-2505. Primers for the PCR amplification according to these methods are derived from V $\alpha$  or V $\beta$  families paired with C $\alpha$  or C $\beta$  primer. Suitable primers for  
10 use in this process are shown in Figure 6. The amplified PCR products are cloned into Bluescript vectors and sequenced. Figure 7 shows the sequences of the variable regions of the  $\alpha$  and  $\beta$  chains of the TCRs recovered from CTLs recovered in mice that had been administered the H7 peptide.

Chimeric molecules similar to those described hereinabove for clone 4 and as  
15 set forth in Figures 1 and 2 were prepared from the amplified sequences of the H7-specific RR functionality is assayed by transfecting MD45.27 and testing for the production of IL-2 as described hereinabove.

A preferred vector for the insertion of the modified sequences, pBJ1Neo with a polylinker insertion site is shown in Figure 8. The host vector, pBJ1Neo is described in  
20 \_\_\_\_\_, *Mol Cell Biol* (1988) 8:466; the polylinker is described by \_\_\_\_\_, *Science* (1990) 249:677.

The dimer and single chain constructs were transfected into 27J cells and the cells measured for production of IL-2 in the presence of JA<sup>2</sup> cells plus H7 peptide. As shown in Figure 9, all transfectants produced with the H7 specific TCR derivatives  
25 produced IL-2. 27J cells without these constructs did not produce IL-2 in response to the JA2 cells and peptide, and none of the cells produced IL-2 in response to JA2 cells alone.

Finally, Figure 10 shows the production of IL-2 by these four constructs transfected into 27J cells in response to HER 2/neu derived peptides and cells

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presenting such peptides. Again, all four constructs rendered the transfected cells responsive.

#### Example 4

##### 5                    Preparation of T cells Expressing TCR and its Derivatives

Human PBL that are CD8<sup>+</sup> are transduced with the chimeric constructs described above using the LXS<sub>N</sub> and LXS<sub>H</sub> retroviral vectors (Hock, R.A. *et al. Nature* (1986) 320:275) and the technique of Anderson, W.F. *Science* (1992) 256:808. The  $\beta$  chimeric gene is inserted into the LXS<sub>H</sub> retroviral vector which confers

10 Hygromycin B resistance and  $\alpha$  chimeric gene in LXS<sub>N</sub> retroviral vector which confers neomycin resistance; thus selection of T lymphocytes expressing both the V $\alpha$ / $\zeta$  and V $\beta$ / $\zeta$  can be recovered. Recombinant retrovirus-producing cell lines are generated by transfection of the vectors into the Ecotropic packaging cell line GP+E86 and the ecotropic virus produced by these cells is used to infect the amphotropic packaging cell

15 line PA317. PA317 clones that produce helper virus free from amphotropic L(V $\alpha$ / $\zeta$ )S<sub>N</sub> and L(V $\beta$ / $\zeta$ )S<sub>H</sub> virus are obtained by selection in G418 or Hygromycin B-containing medium. Clones yielding the highest titer of virus are used to transduce T lymphocytes that have been incubated with anti-CD3 and recombinant IL-2. Similarly, the single-chain TCR is inserted into LXS<sub>N</sub> retroviral vector and introduced similarly.

20                    The resulting transformed human CD8<sup>+</sup>-PBL are tested for cytotoxic activity *in vitro* against tumor cells and then *in vivo* in SCID mice that have received tumor cells displaying the relevant TAA.

- 14 -

Claims

1. A method to prepare an isolated nucleic acid molecule comprising a nucleotide sequence encoding at least one of the variable regions of the  $\alpha$  and  $\beta$  chains  
5 of a non-human TCR which TCR is human HLA-restricted and specific for a tumor-associated antigen, which method comprises  
cloning or amplifying a nucleic acid molecule containing said encoding nucleotide sequence from cytotoxic T lymphocytes (CTL) prepared by a method which comprises  
10 immunizing a transgenic non-human vertebrate which is modified so as to express at least one human HLA antigen with said tumor-associated antigen (TAA) so as to effect the production in said mouse of cytotoxic T lymphocytes which display human HLA-restricted TCR specific for said TAA and which contain nucleic acid molecules comprising nucleotide sequences encoding said variable regions of the  $\alpha$  and  
15  $\beta$  chains of said TCR, and  
recovering said CTL.
2. The method of claim 1 wherein said HLA antigen is a A2.
- 20 3. The method of claim 1 wherein said non-human vertebrate is a mouse.
4. The method of claim 3 wherein said amplifying is effected by a polymerase chain reaction using primers derived from murine TCR.
- 25 5. The method of claim 4 wherein said primers are essentially as set forth in Figure 6.
6. An isolated nucleic acid molecule which comprises a nucleotide sequence encoding a variable region of a non-human TCR  $\alpha$  or  $\beta$  peptide wherein said  
30 TCR is human HLA-restricted and specific for a tumor-associated antigen.



- 15 -

7. The nucleic acid molecule of claim 6 which comprises the  $\alpha$  or  $\beta$  variable region of the said TCR fused to the  $\zeta$  region of CD3, CD8 or CD16.

5 8. The nucleic acid molecule of claim 7 wherein said  $\zeta$  region is that of human CD3, CD8 or CD16.

9. The nucleic acid molecule wherein said non-human TCR is murine.

10 10. The nucleic acid molecule of claim 6 wherein said nucleotide sequence encodes a single-chain TCR.

11. The nucleic acid molecule of claim 10 wherein said single-chain TCR consists of the variable  $\alpha$  region fused to variable  $\beta$  region by a flexible linker and said  
15  $\beta$  region is fused to a  $\zeta$  region.

12. The nucleic acid molecule of claim 11 wherein said flexible linker is of the formula  $(\text{Gly}_4\text{Ser}_3)_3$ .

20 13. The nucleic acid molecule of claim 11 wherein said  $\zeta$  chain is that of CD3, CD8 or CD16.

14. The nucleic acid molecule of claim 13 wherein the  $\zeta$  chain is derived from human CD3, CD8 or CD16.

25 15. A recombinant expression system which expression system comprises the nucleotide sequence of claim 6 operatively linked to control sequences for effecting its expression in a host cell.

- 16 -

16. A recombinant host cell modified to contain the expression system of claim 15.

17. The recombinant cells of claim 16 which are T cells.

5

18. A method to obtain cells which display TCR or a functional derivative thereof at their surface, said TCR or derivative being human HLA-restricted and specific for a tumor-associated antigen, which method comprises culturing the cells of claim 16 under conditions wherein said nucleotide sequence is expressed and said TCR or derivative is displayed at the surface.

10

19. Recombinant cells displaying a TCR receptor or derivative thereof at their surface wherein said TCR or derivative is human HLA-restricted and specific for a tumor-associated antigen prepared by the method of claim 18.

15

20. A method to identify antigens associated with a tumor which method comprises contacting said tumor or a fraction thereof with the cells of claim 19 under conditions wherein said tumor or fraction is lysed only if said tumor displays the TAA for which said TCR or derivative is specific.

20

21. A method to effect treatment of a tumor in a human, wherein said tumor is characterized by a specific tumor-associated antigen (TAA) which method comprises administering to said human subject peripheral blood cells from said subject which have been modified to contain an expression system for a nucleotide sequence which encodes a TCR or derivative thereof which is human HLA-restricted and specific for said TAA.

25

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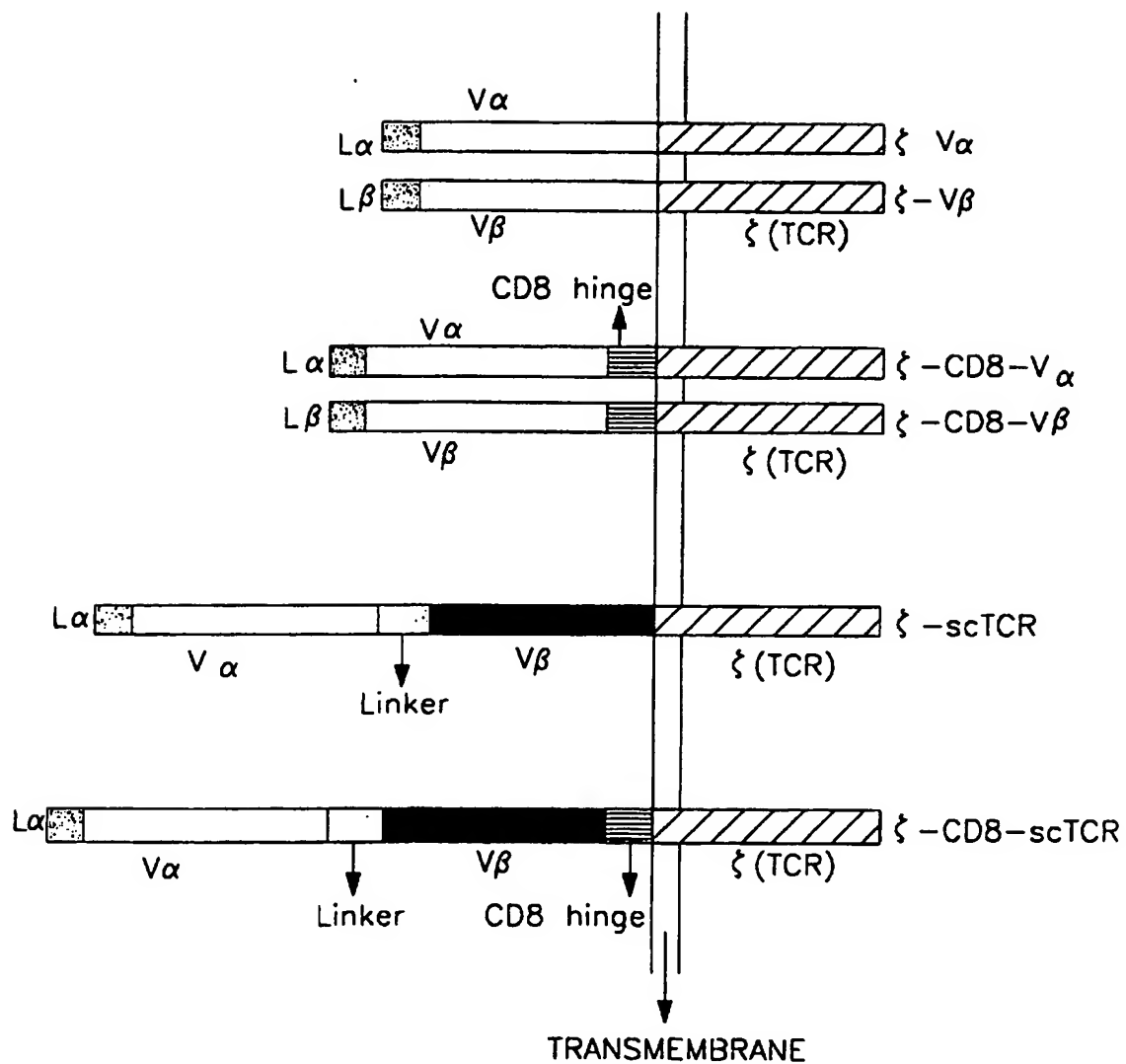


FIG. 1

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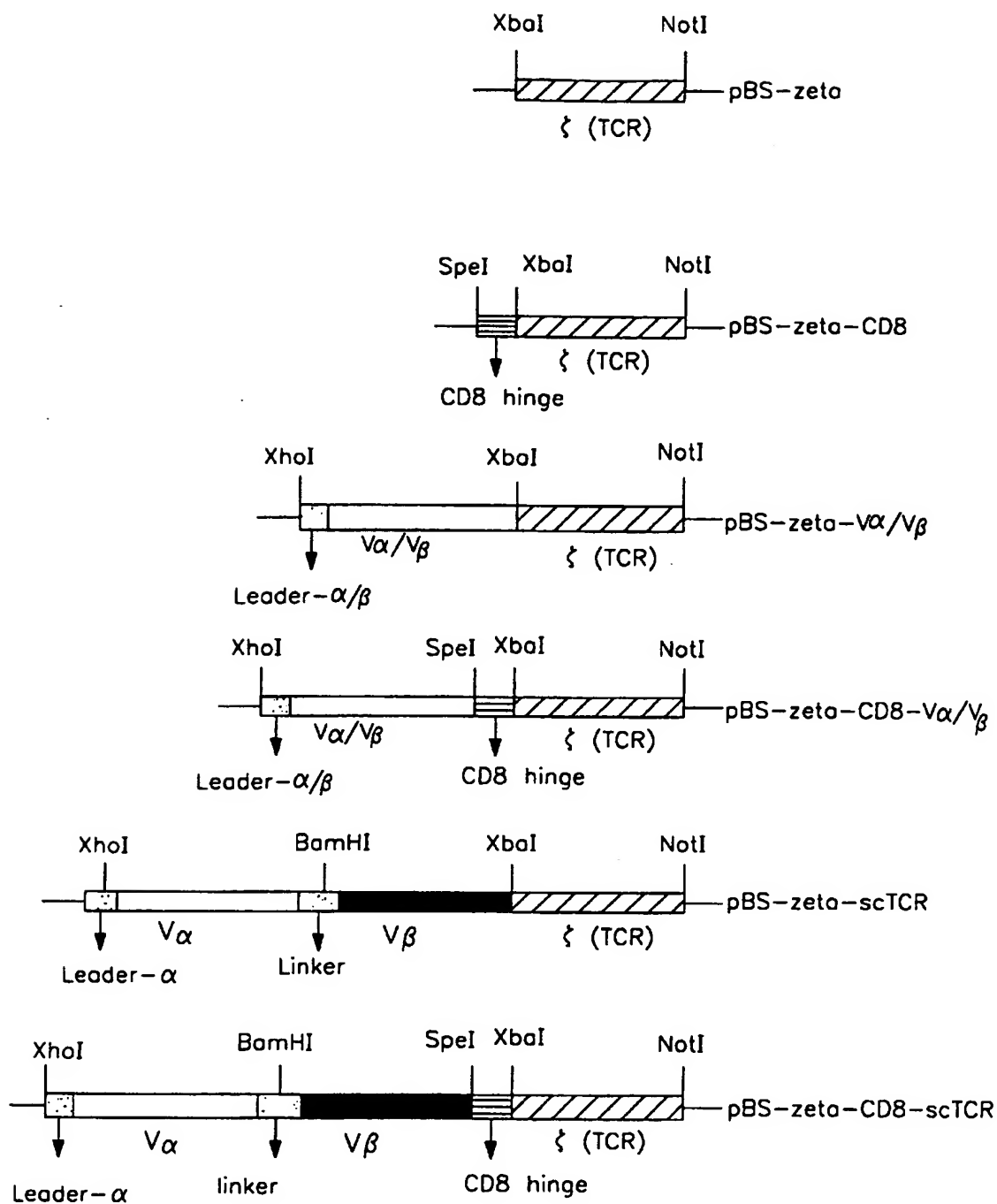


FIG. 2

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L $\alpha$																	
XhoI	9	18	27	36	45	54											
CTC	GAG	ATG	CAG	AGG	AAC	CTG	GGA	GCT	GTG	CTG	GGG	ATT	CTG	TGG	GTG	CAG	ATT
L	E	M	Q	R	N	L	G	A	V	L	G	I	L	W	V	Q	I
TGC	TGG	63	AAA	GAA	72	CAA	GTG	81	CAG	AGT	90	GCA	TCC	99	GTT	CTG	108
C	W	L	K	E	Q	Q	V	Q	Q	S	P	A	S	L	V	L	Q
GAG	GGG	117	GAG	AAC	GCA	126	GAG	CTC	CAG	135	TGT	AGC	TTT	144	TCC	ATC	TTT
E	G	E	N	A	E	L	Q	C	S	F	S	I	F	T	N	Q	V
CAG	TGG	171	TTT	TAC	CAA	180	CGT	CCT	GGG	189	GGA	AGA	CTC	198	GTC	AGC	CTG
Q	W	F	Y	Q	R	P	G	G	R	L	V	S	L	L	Y	N	P
TCT	GGG	225	ACA	AAG	CAG	234	AGT	GGG	AGA	243	CTG	ACA	TCC	252	ACA	ACA	GTC
S	G	T	K	Q	S	G	R	L	T	S	T	T	V	I	K	E	R
CGC	AGC	279	TCT	TTG	CAC	288	ATT	TCC	TCC	297	TCC	CAG	ATC	306	ACA	GAC	TCA
R	S	S	L	H	I	S	S	S	Q	I	T	D	S	G	T	Y	L
TGT	GCC	333	TCA	AAT	TCT	342	GGA	GGA	AGC	351	AAT	GCA	AAG	360	CTA	ACC	TTC
C	A	S	N	S	G	G	S	N	A	K	L	T	F	G	K	G	T
AAA	CTC	387	TCT	GTT	AAA	396	TCA	GGT	GGC	405	GGA	GGG	TCT	414	GGC	GGG	GGT
K	L	S	V	K	S	G	G	G	G	S	G	G	G	G	G	S	G
LINKER																	
GGA	GGC	441	TCA	GAG	GCT	450	GCA	GTC	ACC	459	CAA	AGC	CCA	468	AGA	AAC	AAG
G	G	S	E	A	A	V	T	Q	S	P	R	N	K	V	A	V	T
GGA	GGA	495	AAG	GTG	ACA	504	TTG	AGC	TGT	513	AAT	CAG	ACT	522	AAT	AAC	CAC
G	G	K	V	T	L	S	C	N	Q	T	N	N	H	N	N	M	Y
TGG	TAT	549	CGG	CAG	GAC	558	ACG	GGG	CAT	567	GGG	CTG	AGG	576	CTG	ATC	CAT
W	Y	R	Q	D	T	G	H	G	L	R	L	I	H	Y	S	Y	G

FIG. 3A

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GCT	GGC	603	AGC	ACT	GAG	612	AAA	GGA	GAT	621	ATC	CCT	GAT	630	GGA	TAC	AAG	639	GCC	TCC	AGA	648	CCA
A	G	S	T	E	K	G	D	I	P	D	G	Y	K	A	S	R	P						
AGC	CAA	657	GAG	AAC	TTC	666	TCC	CTC	ATT	675	CTG	GAG	TTG	684	GCT	ACC	CCC	693	TCT	CAG	ACA	702	TCA
S	Q	E	N	F	S	L	I	L	E	L	A	T	P	S	Q	T	S						
GTG	TAC	711	TTC	TGT	GCC	720	AGC	GGT	GAG	729	ACA	GGG	ACC	738	AAC	GAA	AGA	747	TTA	TTT	TTC	756	GGT
V	Y	F	C	A	S	G	E	T	G	T	N	E	R	L	F	F	G						Vβ
CAT	GGA	765	ACC	AAG	CTG	774	TCT	GTC	CTG	783	ACT	AGT	AAC	792	TCC	ATC	ATG	801	TAC	TTC	AGC	810	CAC
H	G	T	K	L	S	V	L	T	S	N	S	I	M	Y	F	S	H						
TTC	GTG	819	CCG	GTC	TTC	828	CTG	CCA	GCG	837	AAG	CCC	ACC	846	ACG	ACG	CCA	855	GCG	CCG	CGA	864	CCA
F	V	P	V	F	L	P	A	K	P	T	T	T	P	A	P	R	P						CD8 HINGE
CCA	ACA	873	CCG	GCG	CCC	882	ACC	ATC	GCG	891	TGG	CAG	CCC	900	CTG	TCC	CTG	909	GCG	CCA	TCT	918	AGT
P	T	P	A	P	T	I	A	S	Q	P	L	S	L	R	P	S	S						
<u>AbaI</u>	AGA	927	GAT	CCC	AAA	936	CTC	TGC	TAC	945	CTG	CTG	GAT	954	GGA	ATC	CTC	963	TTC	ATC	TAT	972	GGT
S	R	D	P	K	L	C	Y	L	L	D	G	I	L	F	I	Y	G						
GTC	ATT	981	CTC	ACT	GCC	990	TTG	TTC	CTG	999	AGA	GTG	AAG	1008	TTC	AGC	AGG	1017	AGC	GCA	GAC	1026	GCC
V	I	L	T	A	L	F	L	R	V	K	F	S	R	S	A	D	A						
CCC	GCG	1035	TAC	CAG	CAG	1044	GCG	CAG	AAC	1053	CAG	CTC	TAT	1062	AAC	GAG	CTC	1071	AAT	CTA	GGA	1080	CGA
P	A	Y	Q	Q	G	Q	N	Q	L	Y	N	E	L	N	L	G	R						
AGA	GAG	1089	GAG	TAC	GAT	1098	GTT	TTG	GAC	1107	AAG	AGA	CGT	1116	GCG	CGG	GAC	1125	CCT	GAG	ATG	1134	GGG
R	E	E	Y	D	V	L	D	K	R	R	G	R	D	P	E	M	G						Z CHAIN

FIG. 3B

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GGA	AAG	CCG	AGA	AGG	AAG	AAC	CCT	CAG	GAA	GGC	CTG	TAC	AAT	GAA	CTG	CAG	AAA
G	K	P	R	R	K	N	P	Q	E	G	L	Y	N	E	L	Q	K
GAT	AAG	ATG	GCG	GAG	GCC	TAC	AGT	GAG	ATT	GGG	ATG	AAA	GGC	GAG	CGC	CGG	AGG
D	K	M	A	E	A	Y	S	E	I	G	M	K	G	E	R	R	R
GGC	AAG	GGG	CAC	GAT	GGC	CTT	TAC	CAG	GGT	CTC	AGT	ACA	GCC	ACC	AAG	GAC	ACC
G	K	G	H	D	G	L	Y	Q	G	L	S	T	A	T	K	D	T
TAC	GAC	GCC	CTT	CAC	ATG	CAG	GCC	CTG	CCC	CCT	CGC	TAA	GCG	GCC	GCC	ACC	GCG
Y	D	A	L	H	M	Q	A	L	P	P	R	*	A	A	A	T	A

NOT I  
STOP

FIG. 3C

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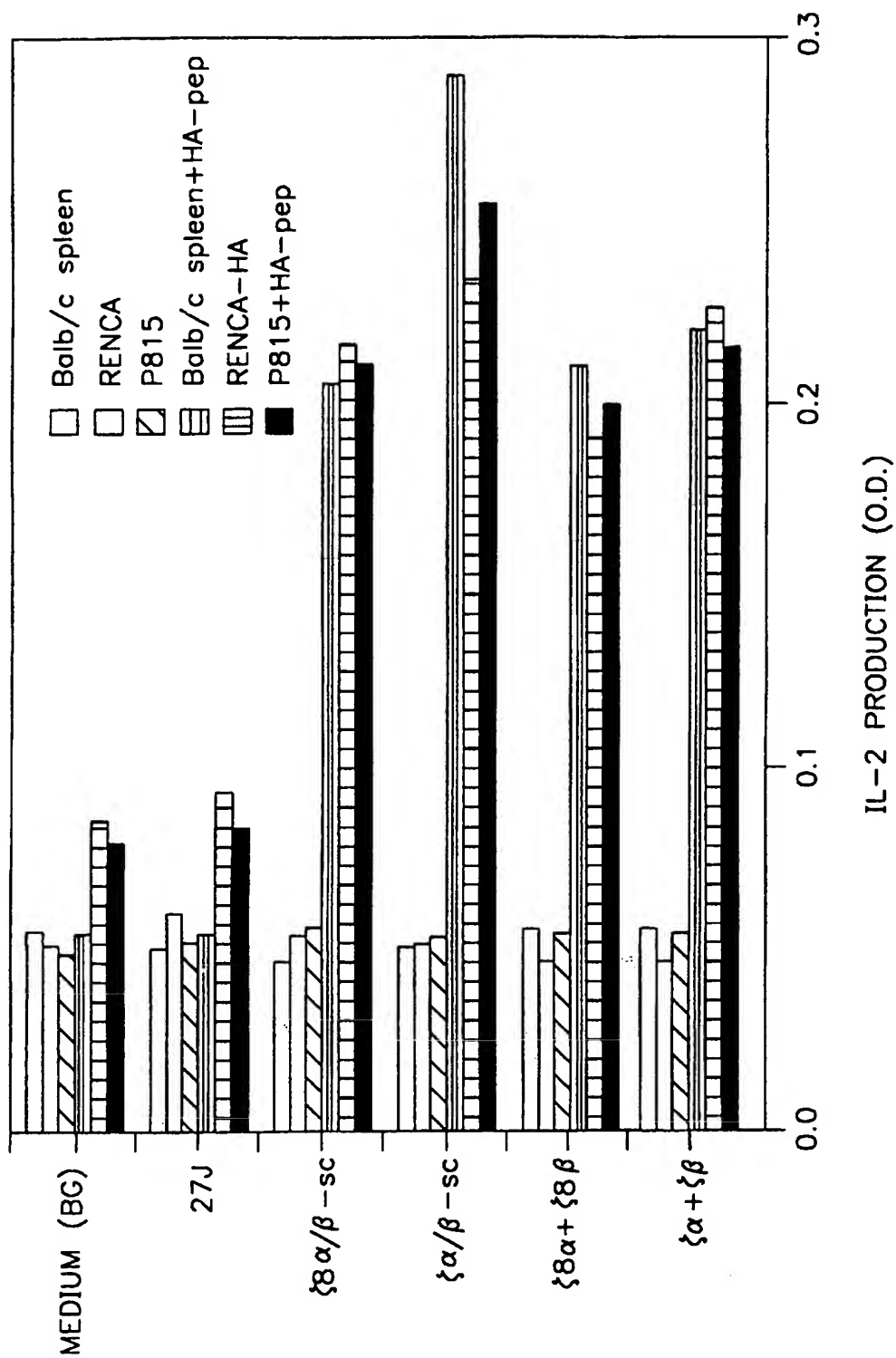


FIG. 4



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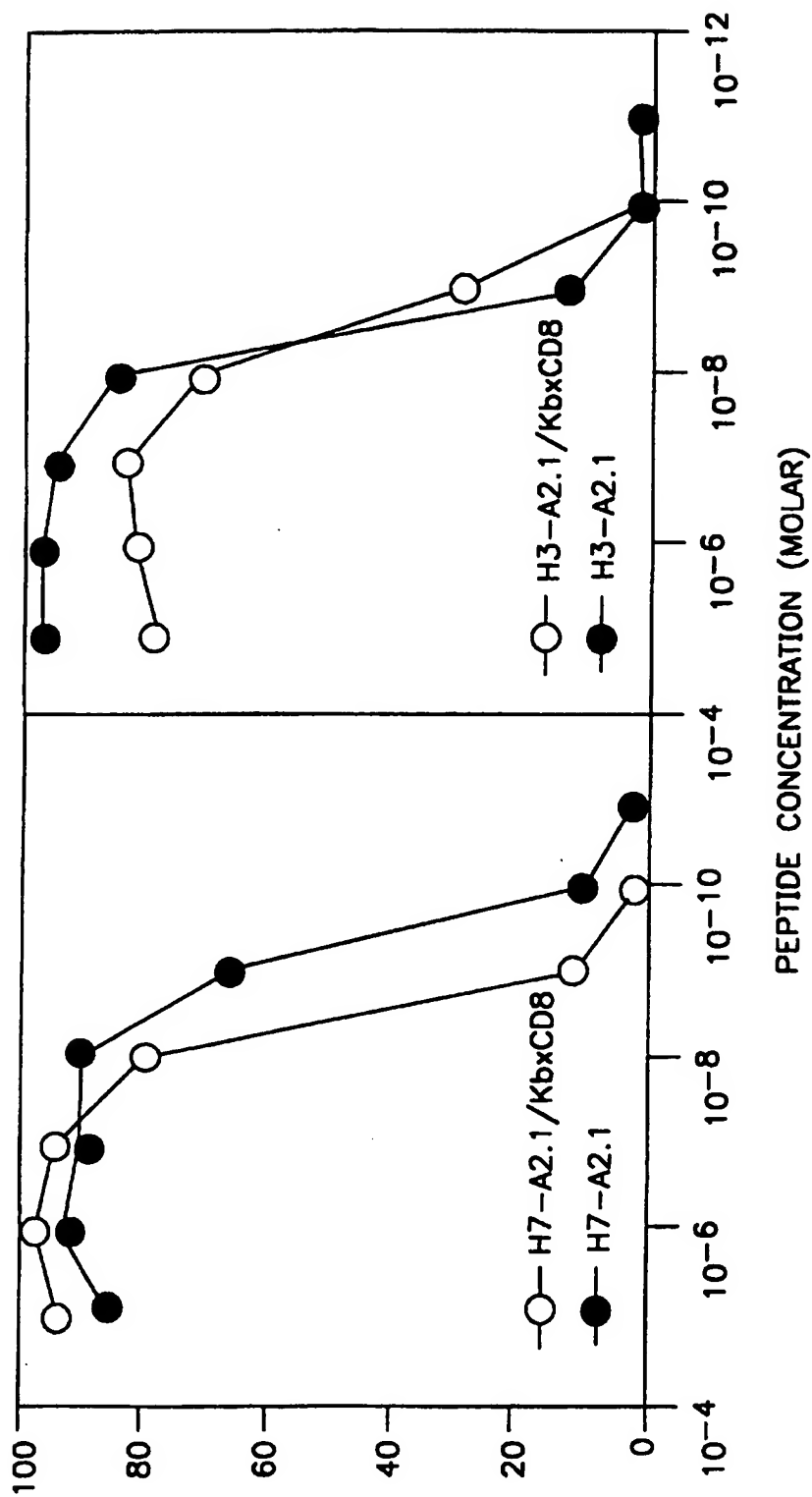


FIG. 5

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## Alpha Groups

Va1	CCC	AAG	GCA	CTG	ATG	TTC	ATC	TTC		
Va2	TGA	GAC	AAA	GTC	CCC	AAT	CTC	TGA	CAG	
Va3	CTG	CAG	CTG	CTC	CTC	AAG	TAC	TAT	TC	
Va4.1.2.3	TCC	CGG	AGA	AGG	TCC	ACA	GTT	CCT	CTT	T
Va4.4	GAA	GCA	GCA	GAG	GGT	TTG	AAG	CCA	CAT	AC

<b>2.</b>									
Vα5	GGC	AGG	TCT	TCA	GTT	GCT	TAT	GAA	GGT
Vα6	GGT	TCC	TCT	TCA	GGG	TCC	AGA	ATA	TGT
Vα7	GCG	AAG	AAC	TCA	CCC	TGG	ACT	GTT	CAT
Vα8	GAG	CTC	CAC	AGA	CAA	CAA	GAG	GAC	CGA
Vα9	GAG	CTG	CGA	CGT	TCC	TTA	GTG	ACT	GTG

<b>3.</b>										
Vα10	CCT	CGT	CAG	CCT	GTT	GTC	CAA	TCC	TTC	TGG
Vα11	CAG	CCT	CAT	CAA	TCT	GTT	CTA	CTT	GGC	T
Vα12	CCA	CCA	GGG	ACC	ACA	GTT	TAT	CAT	TCA	A
Vα14	ACC	TGG	AGA	GAA	TCC	TAA	GCT	CAT	CAT	
Vα15	AGG	TCT	TGT	GTC	CCT	GAC	AGT	CCT	GGT	T

4.													
Vα <sub>16</sub>	CAA	GCA	AAC	ACT	GTA	GTG	CAG	AGC	CCT	TCC			
Vα <sub>17</sub>	CAA	GAT	ATC	CAT	AAC	TGC	CCT	ACA	G				
Vα <sub>18</sub>	GTG	TAT	GAA	ACC	CAG	GAC	AGT	TCT	TAC				
Vα <sub>19</sub>	CCG	TAT	TTC	TTT	CTT	ATG	TTG	TTT	TGG	AT			
Vα <sub>20</sub>	CAA	AGC	TCT	CCA	TCG	CTG	ACT	GTT	CAA	G			

## Beta Groups

Vβ1	ATC	TAA	TCC	TGG	GAA	GAG	CAA	AT
Vβ2	GGC	GTC	TGG	TAC	CAC	GTG	GTC	AA
Vβ3	GTG	AAA	GGG	CAA	GGA	CAA	AAA	GC
Vβ4	GAT	ATG	CGA	ACA	GTG	TCT	AGG	C
Vβ5.1	ACA	TAA	TCA	AAG	GAA	AGG	GAG	AA

2.	TCC	TGA	TTG	GTC	AGG	AAG	GGC	AA
V $\beta$ 6	TAC	CTG	ATC	AAA	AGA	ATG	GGA	GA
V $\beta$ 7	ATA	ACC	ATG	ACA	ATA	TGT	ACT	GG
V $\beta$ 8.1	ATA	ACC	ACA	ACA	ACA	TGT	ACT	GG
V $\beta$ 8.2	ATA	GCC	ACA	ACT	ACA	TGT	ACT	GG
V $\beta$ 8.3								

3.								
Vβ9	AGC	TTG	CAA	GAG	TTG	GAA	AAC	CA
Vβ10	GAT	TAT	GTT	TAG	CTA	CAA	TAA	TA
Vβ11	ACA	AGG	TGA	CAG	GGA	AGG	GAC	AA
Vβ12	ACC	TAC	AGA	ACC	CAA	GGA	CTC	AG
Vβ13	CAG	TTG	CCC	TCG	GAT	CGA	TTT	TC

4. 

V $\beta$ 14	GCC	GAG	ATC	AAG	GCT	GTG	GGC	AG
V $\beta$ 15	AGA	ACC	ATC	TGT	AAG	AGT	GGA	AC
V $\beta$ 16	CAT	CAA	ATA	ATA	GAT	ATG	GGG	CA
V $\beta$ 17	GTA	GTC	CTG	AAA	AAG	GGC	ACA	CT
V $\beta$ 18	CAT	CTG	TCA	AAG	TGG	CAC	TTC	A

**FIG. 6**

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ATG	AAA	<sup>9</sup> TCC	TTG	AGT	<sup>18</sup> GTT	TCC	CTA	<sup>27</sup> GTG	GTC	CTG	<sup>36</sup> TGG	CTC	CAG	<sup>45</sup> TTA	AAC	TGG	<sup>54</sup> GTG
Met	Lys	Ser	Leu	Ser	Val	Ser	Leu	Val	Val	Leu	Trp	Leu	Gln	Leu	Asn	Trp	Val
CAG	AGC	<sup>63</sup> CAG	CAG	AAG	<sup>72</sup> GTG	CAG	CAG	<sup>81</sup> AGC	CCA	GAA	<sup>90</sup> TCC	CTC	AGT	<sup>99</sup> GTC	CCA	GAG	<sup>108</sup> GGG
Gln	Ser	Gln	Gln	Lys	Val	Gln	Gln	Ser	Pro	Glu	Ser	Leu	Ser	Val	Pro	Glu	Gly
GGC	ATG	<sup>117</sup> GCC	TCT	CTC	<sup>126</sup> AAC	TGC	ACT	<sup>135</sup> TCA	AGT	GAT	<sup>144</sup> CGC	AAT	TTT	<sup>153</sup> CAG	TAT	TTC	<sup>162</sup> TGG
Gly	Met	Ala	Ser	Leu	Asn	Cys	Thr	Ser	Ser	Asp	Arg	Asn	Phe	Gln	Tyr	Phe	Trp
TGG	TAC	<sup>171</sup> AGA	CAG	CAT	<sup>180</sup> TCT	GGA	GAA	<sup>189</sup> GGC	CCC	AAA	<sup>198</sup> GCA	CTG	ATG	<sup>207</sup> TCC	ATC	TTC	<sup>216</sup> TCT
Trp	Tyr	Arg	Gln	His	Ser	Gly	Glu	Gly	Pro	Lys	Ala	Leu	Met	Ser	Ile	Phe	Ser
GAT	GGT	<sup>225</sup> GAC	AAG	AAA	<sup>234</sup> GAA	GGC	AGA	<sup>243</sup> TTC	ACA	GCT	<sup>252</sup> CAC	CTC	AAT	<sup>261</sup> AAG	GCC	AGC	<sup>270</sup> CTG
Asp	Gly	Asp	Lys	Lys	Glu	Gly	Arg	Phe	Thr	Ala	His	Leu	Asn	Lys	Ala	Ser	Leu
CAT	GTT	<sup>279</sup> TCC	CTG	CAC	<sup>288</sup> ATC	AGA	GAC	<sup>297</sup> TCC	CAG	CCC	<sup>306</sup> AGT	GAC	TCC	<sup>315</sup> GCT	CTC	TAC	<sup>324</sup> TTC
His	Val	Ser	Leu	His	Ile	Arg	Asp	Ser	Gln	Pro	Ser	Asp	Ser	Ala	Leu	Tyr	Phe
TGT	GCA	<sup>333</sup> GTT	ATG	GAT	<sup>342</sup> TAT	AAC	CAG	<sup>351</sup> GGG	AAG	CTT	<sup>360</sup> ATC	TTT	GGG	<sup>369</sup> CAG	GGT	ACC	<sup>378</sup> AAG
Cys	Ala	Val	Met	Asp	Tyr	Asn	Gln	Gly	Lys	Leu	Ile	Phe	Gly	Gln	Gly	Thr	Lys
TTA	TCT	<sup>387</sup> ATC	AAG	CCC	3'												
Leu	Ser	Ile	Lys	Pro													

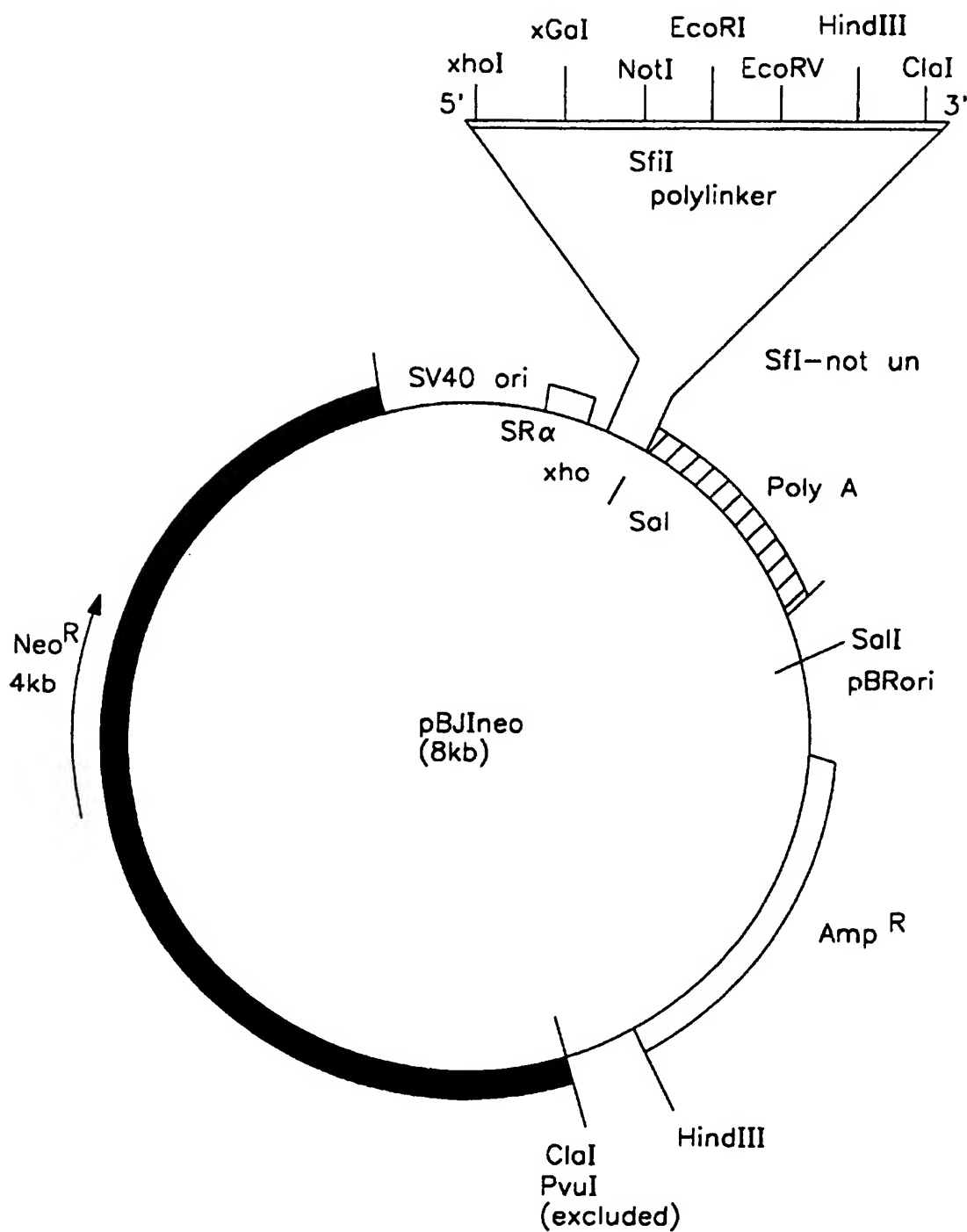
FIG. 7A

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ATG	GGC	TCC	AGA	CTC	TTC	TTT	GTG	GTT	TTG	ATT	CTC	CTG	TGT	GCA	AAA	CAC	ATG
Met	Gly	Ser	Arg	Leu	Phe	Phe	Val	Val	Leu	Ile	Leu	Leu	Cys	Ala	Lys	His	Met
GAG	GCT	GCA	GTC	ACC	CAA	AGT	CCA	AGA	AGC	AAG	GTG	GCA	GTA	ACA	GGA	GGA	AAG
Glu	Ala	Ala	Val	Thr	Gln	Ser	Pro	Arg	Ser	Lys	Val	Ala	Val	Thr	Gly	Gly	Lys
GTG	ACA	TTG	AGC	TGT	CAC	CAG	ACT	AAT	AAC	CAT	GAC	TAT	ATG	TAC	TGG	TAT	CGG
Val	Thr	Leu	Ser	Cys	His	Gln	Thr	Asn	Asn	His	Asp	Tyr	Met	Tyr	Trp	Tyr	Arg
CAG	GAC	ACG	GGG	CAT	GGG	CTG	AGG	CTG	ATC	CAT	TAC	TCA	TAT	GTC	GCT	GAC	AGC
Gln	Asp	Thr	Gly	His	Gly	Leu	Arg	Leu	Ile	His	Tyr	Ser	Tyr	Val	Ala	Asp	Ser
ACG	GAG	AAA	GGA	GAT	ATC	CCT	GAT	GGG	TAC	AAG	GCC	TCC	AGA	CCA	AGC	CAA	GAG
Thr	Glu	Lys	Gly	Asp	Ile	Pro	Asp	Gly	Tyr	Lys	Ala	Ser	Arg	Pro	Ser	Gln	Glu
AAT	TTC	TCT	CTC	ATT	CTG	GAG	TTG	GCT	TCC	CTT	TCT	CAG	TCA	GCT	GTA	TAT	TTC
Asn	Phe	Ser	Leu	Ile	Leu	Glu	Leu	Ala	Ser	Leu	Ser	Gln	Ser	Ala	Val	Tyr	Phe
TGT	GCC	AGC	AGC	GAT	TTC	GCC	GGG	ACA	GGG	GGC	TTC	TAT	GAA	CAG	TAC	TTC	GGT
Cys	Ala	Ser	Ser	Asp	Phe	Ala	Gly	Thr	Gly	Gly	Phe	Tyr	Glu	Gln	Tyr	Phe	Gly
CCC	GGC	ACC	AGG	CTC	ACG	GTT	TCT	3'									
Pro	Gly	Thr	Arg	Leu	Thr	Val	Ser										

FIG. 7B

11 / 13



Ref:

pBJIneo— MCB 8: 466,1988

polylinker— Science,249:677,1990

**FIG. 8**

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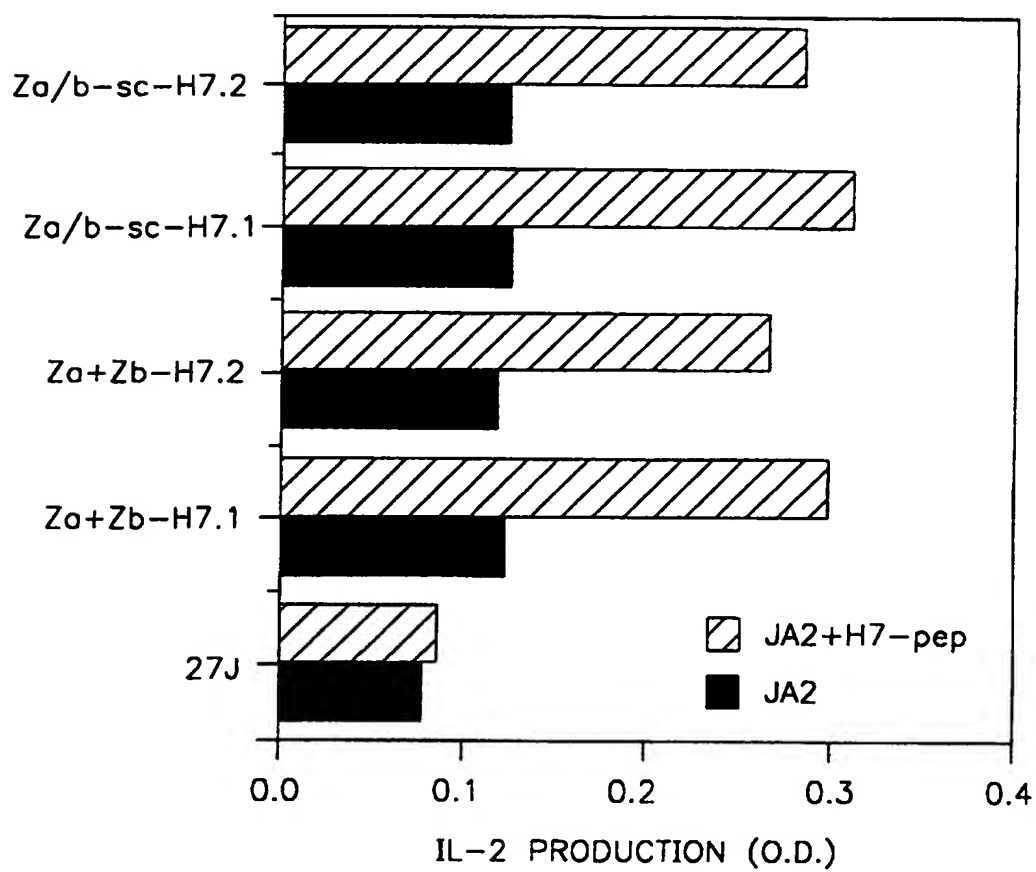


FIG. 9

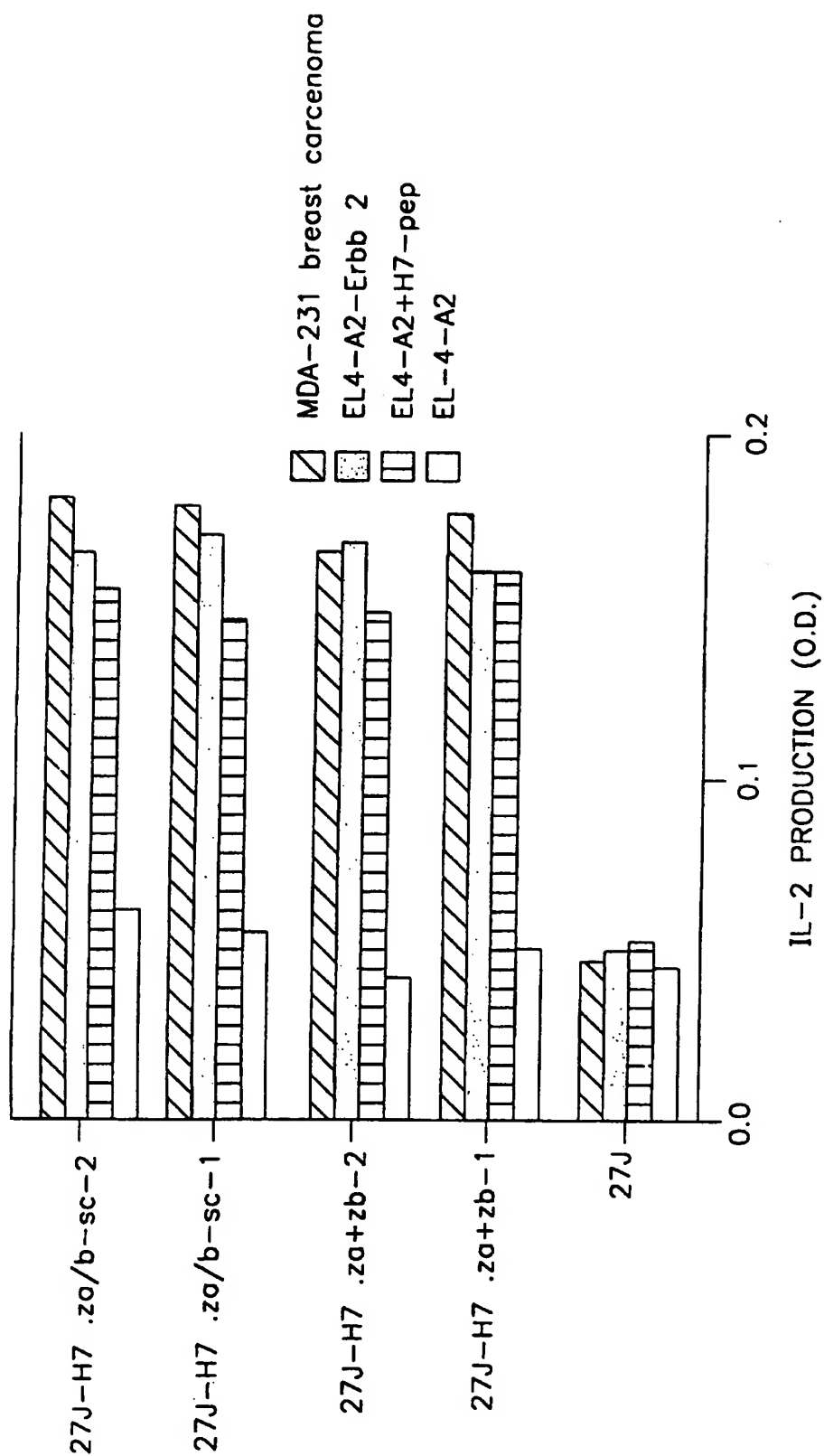


FIG. 10

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/03611

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : 536/23.4, 23.5; 435/6, 91.41, 372.3; 424/277.1

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.4, 23.5; 435/6, 91.41, 372.3; 424/277.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN medline embase scisearch biosis lifesci cancerlit

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	THEOBALD M. et al. Targeting p53 as a general tumor antigen. Proc. Natl. Acad. Sci. USA. December 1995, Vol. 92, pages 11993-11997, see entire document.	1-20
Y	LE A.T. et al. Cytotoxic T cell responses in HLA-A2.1 transgenic mice. J. Immunol. 15 February 1989, Vol. 142, No. 4, pages 1366-1371, see entire document.	1-20
Y	RESSING M.E. et al. Human CTL epitopes encoded by human papillomavirus type 16 E6 and E7 identified through in vivo and in vitro immunogenicity studies of HLA-A*0201-binding peptides. J. Immunol. 01 June 1995, Vol. 154, pages 5934-5943, see entire document.	1-6, 15-20

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	* T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
* A* document defining the general state of the art which is not considered to be of particular relevance	* X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
* E* earlier document published on or after the international filing date	* Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
* L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	* A*	document member of the same patent family
* O* document referring to an oral disclosure, use, exhibition or other means		
* P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

25 APRIL 1997

Date of mailing of the international search report

07 MAY 1997

Name and mailing address of the ISA/US  
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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/03611

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	HOCK R.A. et al. Retrovirus-mediated transfer and expression of drug resistance genes in human haematopoietic progenitor cells. Nature. 20 March 1996, Vol. 320, pages 275-277, see entire document.	6-19
Y	LaFACE D.M. et al. Human CD8 transgene regulation of HLA recognition by murine T cells. J. Exp. Med. November 1995, Vol. 182, pages 1315-1325, see entire document.	1-20
Y	WENTWORTH P.A. et al. Differences and similarities in the A2.1-restricted cytotoxic T cell repertoire in humans and human leukocyte antigen-transgenic mice. Eur. J. Immunol. February 1996, Vol. 26, pages 97-101, especially pages 97-98.	1-20
Y	MAN S. et al. Diversity and dominance among TCR recognizing HLA-A2.1* influenza matrix peptide in human MHC Class I transgenic mice. J. Immunol. November 1994, Vol. 153, pages 4458-4467, especially page 4459 and Figure 5.	4-19
Y	ZISMAN E. et al. Dichotomy between the T and the B cell epitopes of the synthetic peptide (T,G)-A-L*. Eur. J. Immunol. November 1994, Vol. 24, pages 2497-2505, especially Tables 2 and 3.	4-19
A, P	LUSTGARTEN J. et al. Identification of Her-2/Neu CTL epitopes using double transgenic mice expressing HLA-A2.1 and human CD.8. Human Immunology. February 1997, Vol. 52, No. 2, pages 109-118.	1-20
A	MOLLER P. et al. The role of surface HLA-A,B,C molecules in tumour immunity. Cancer Surveys. 1992, Vol. 13, pages 101-127.	1-20

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/03611

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-20

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/03611

## A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

A61K 45/05; C12N 5/10, 15/12; C07H 21/04; C12Q 1/68

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING.

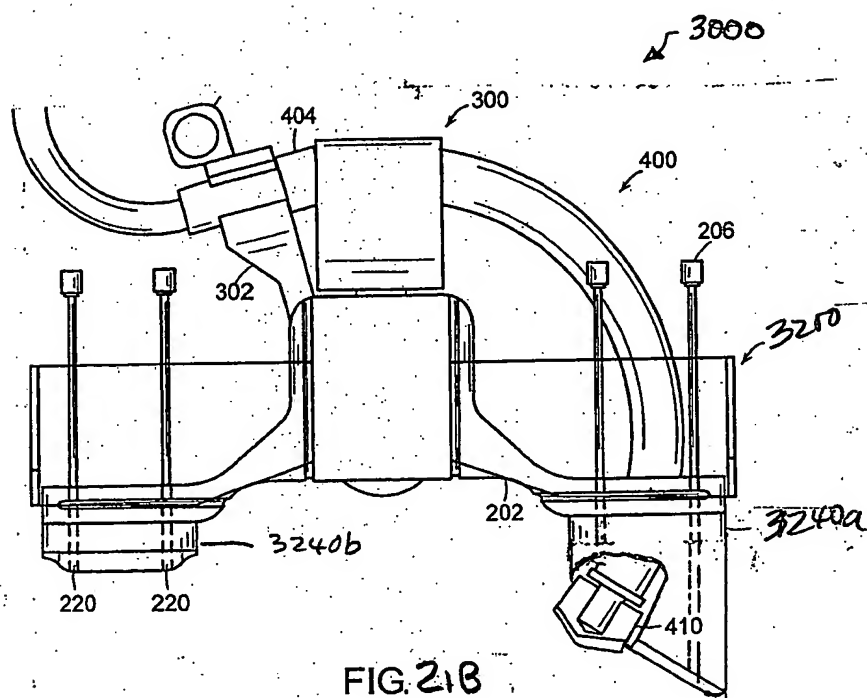
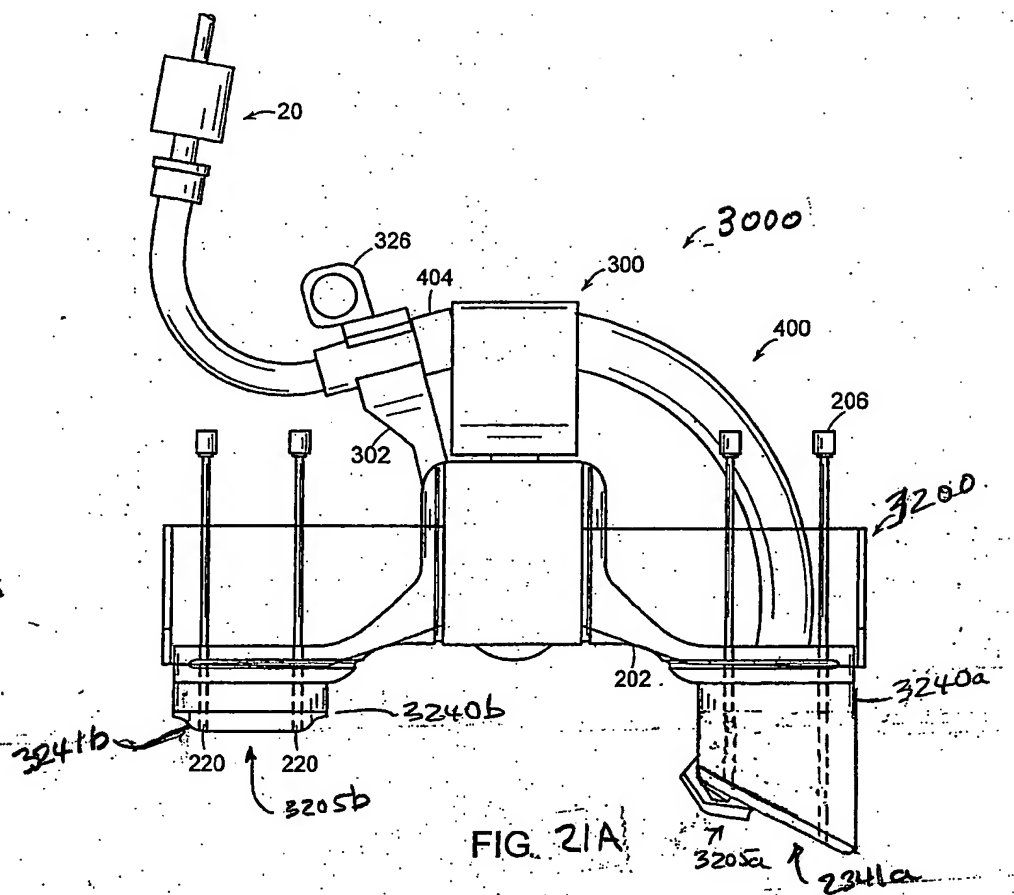
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1.

Group I, claims 1-20, drawn to an isolated TCR nucleic acid, recombinant expression system, recombinant cells with the TCR recombinant expression system and method of use thereof.

Group II, claim 21, drawn to treatment of a human tumor by gene therapy.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The method of treating a patient with a particular tumor using recombinant human cells in Group II is different from methods of diagnosing a tumor in Group I. Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.



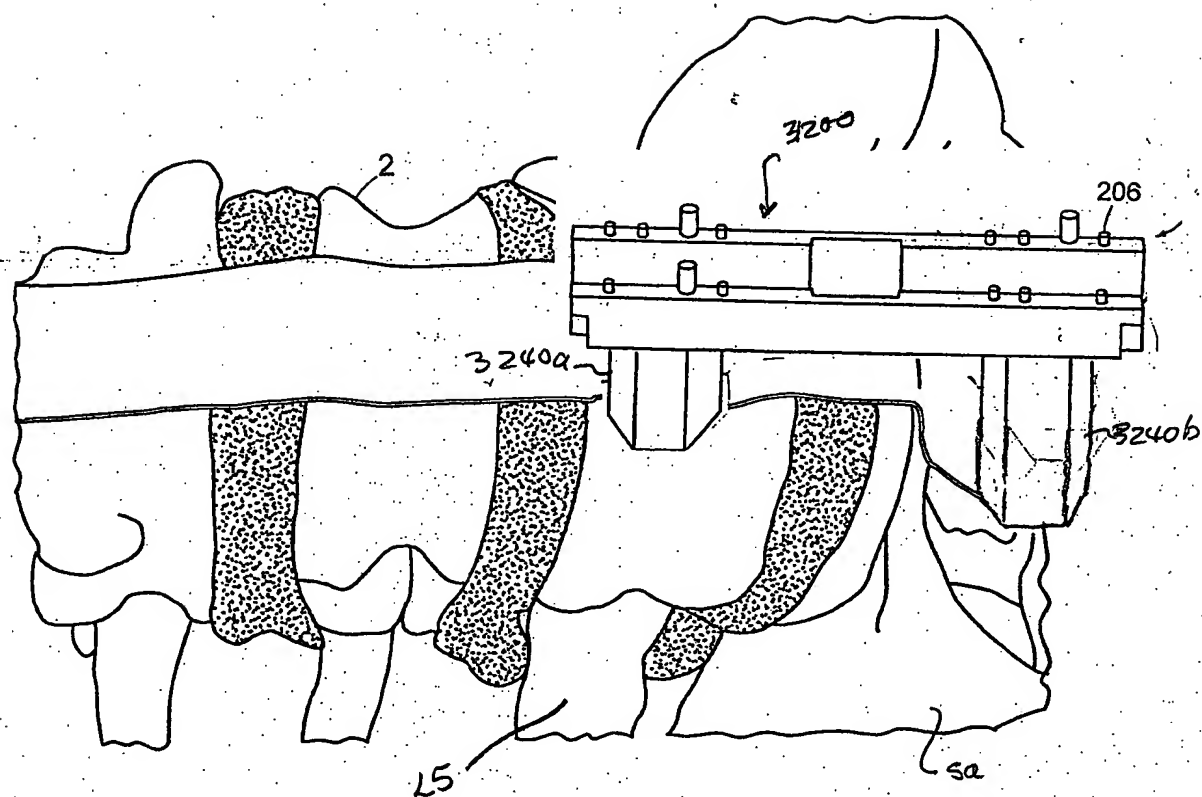
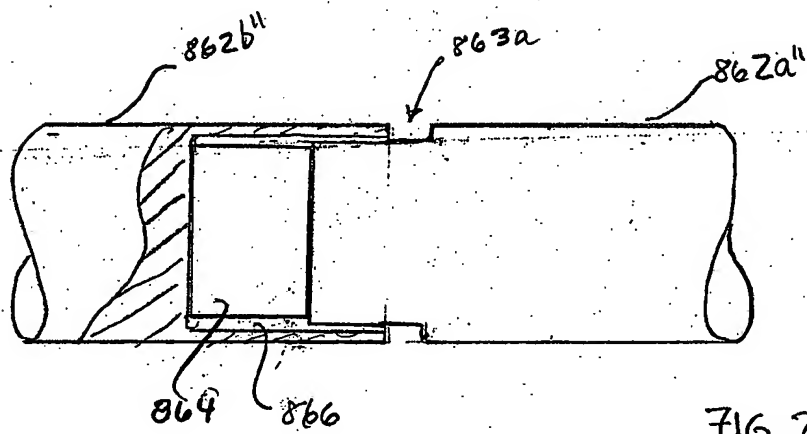
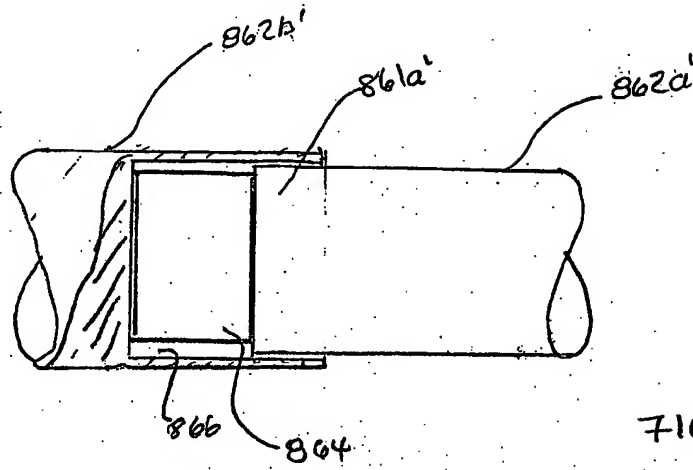
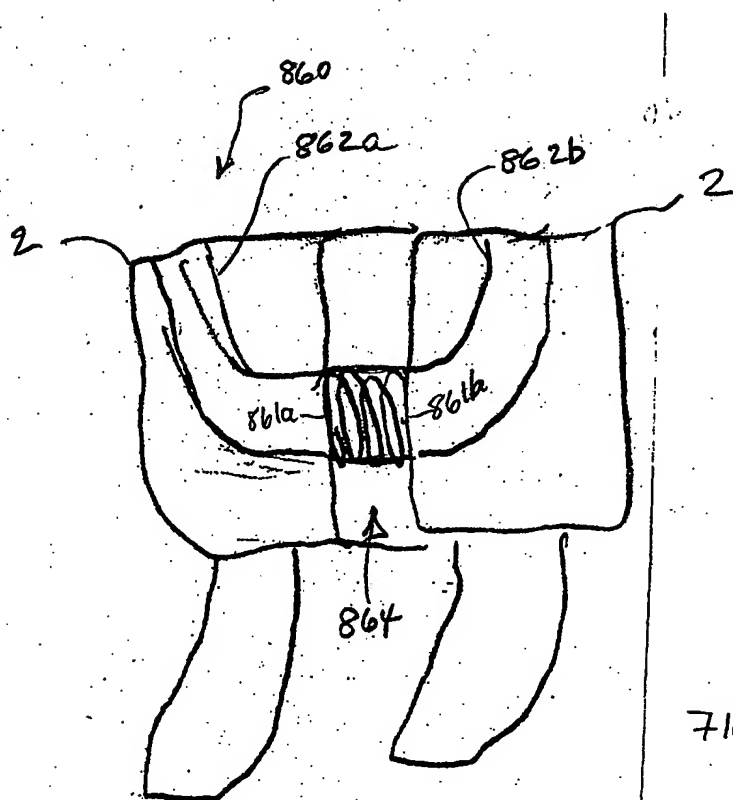
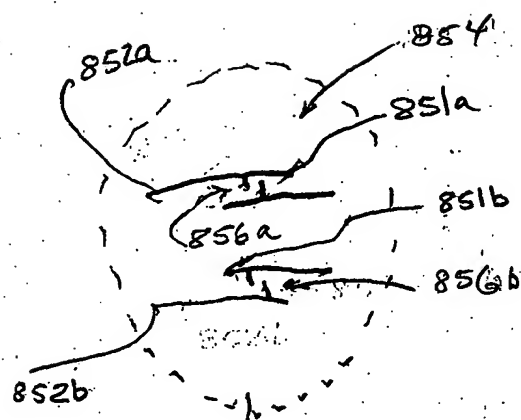
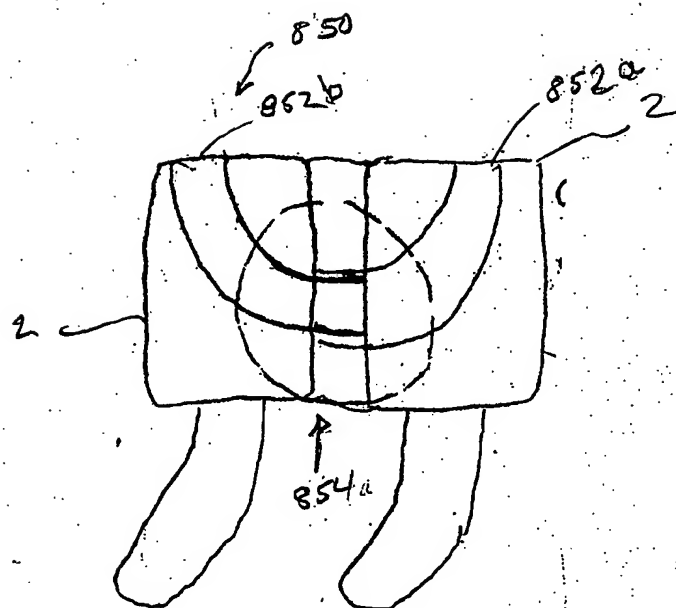


FIG. 21C





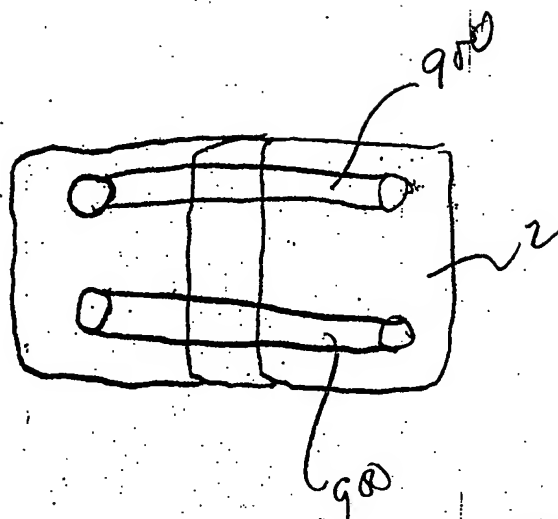


FIG. 24A

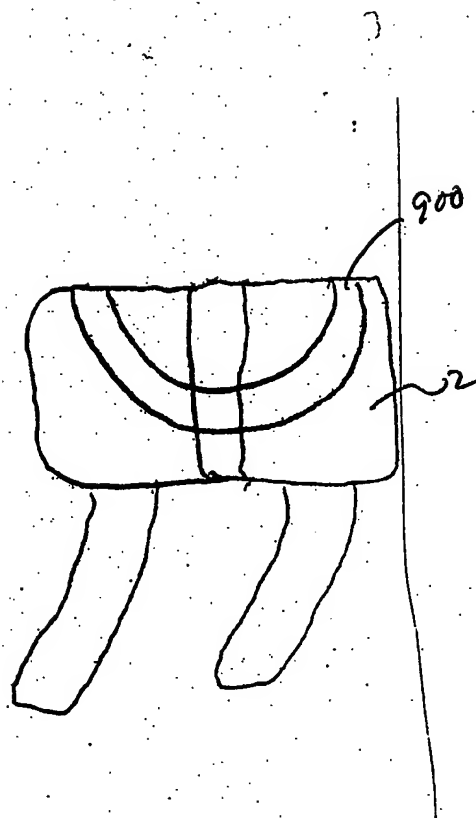


FIG. 24B



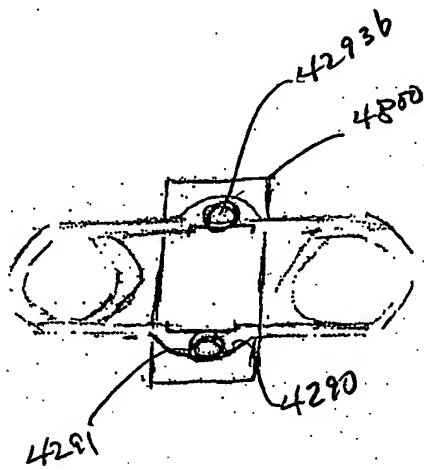


FIG. 25A

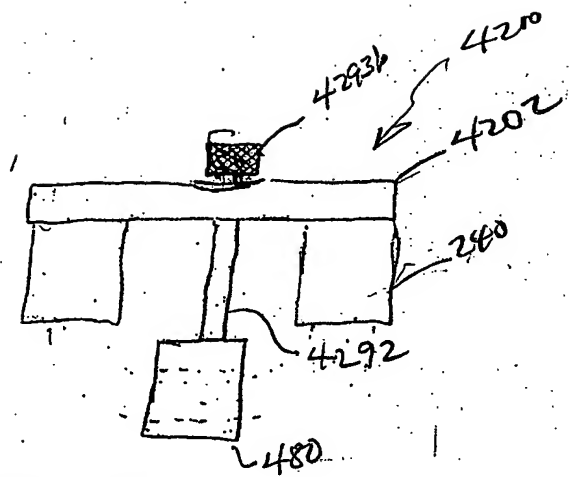
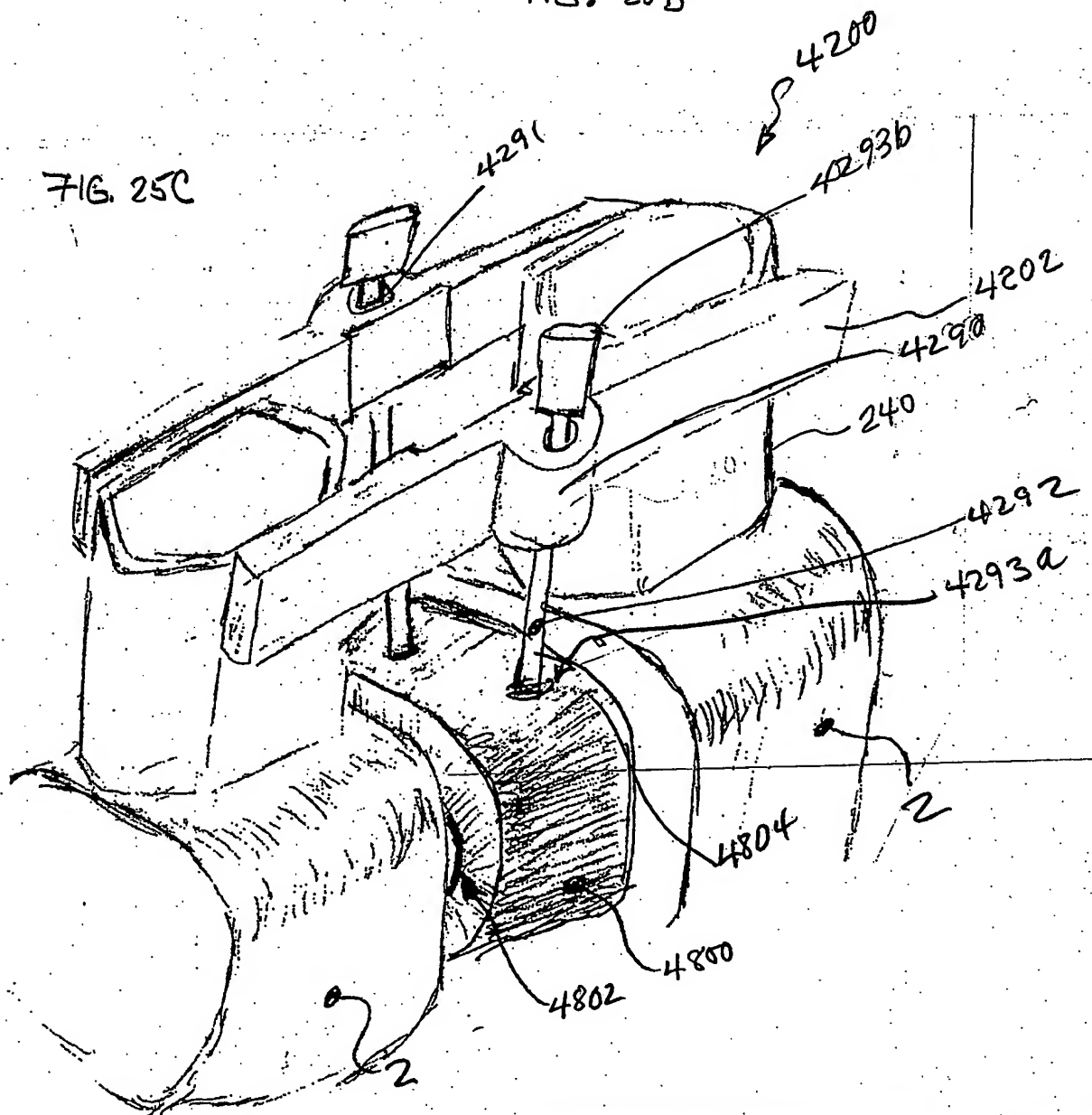


FIG. 25B



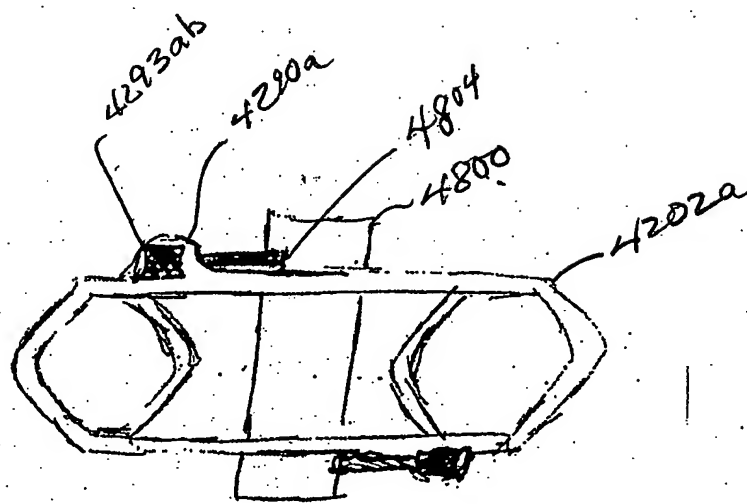


FIG. 26 A

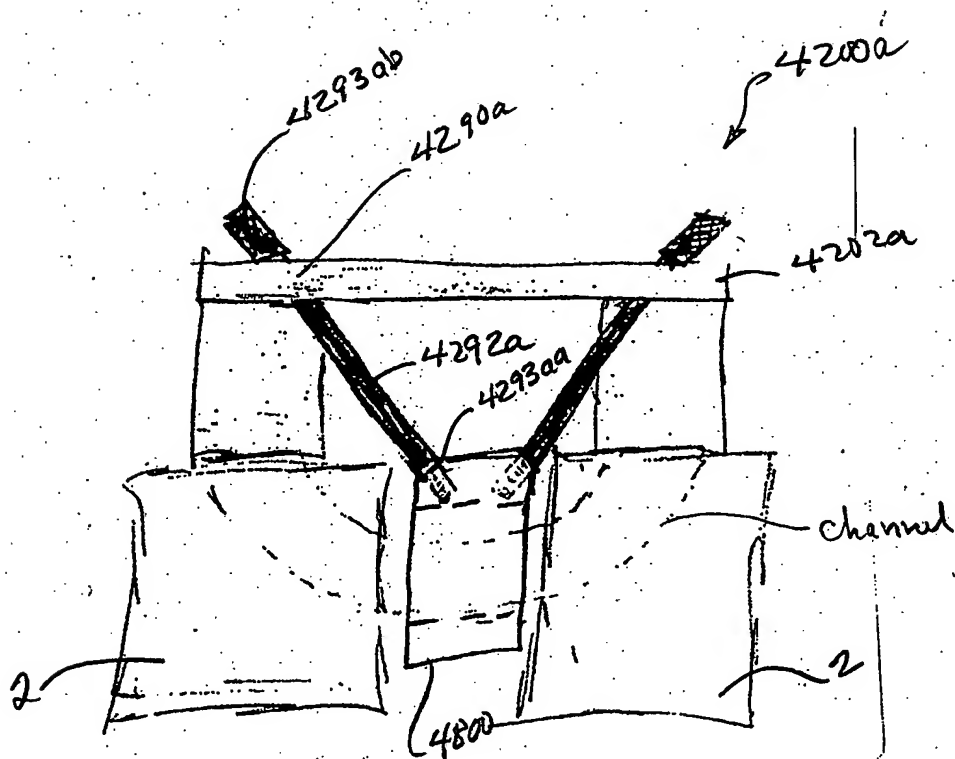


FIG. 26 B

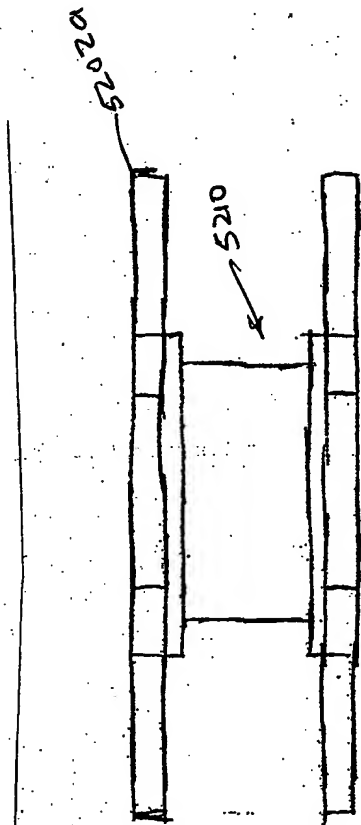


FIG. 27A

5200a

5200a

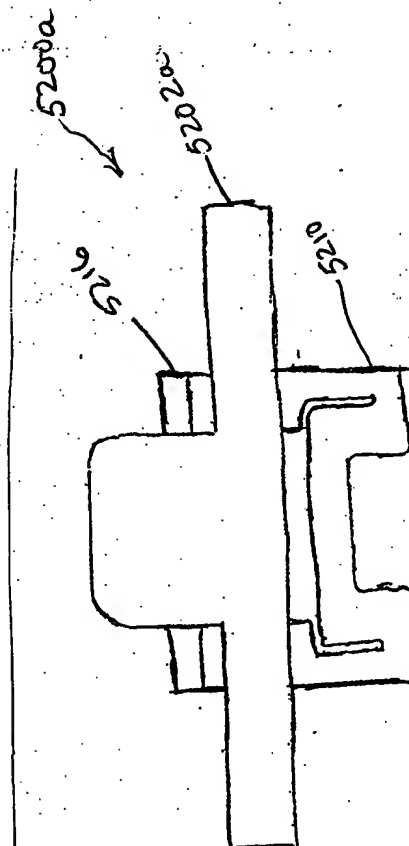


FIG. 27B

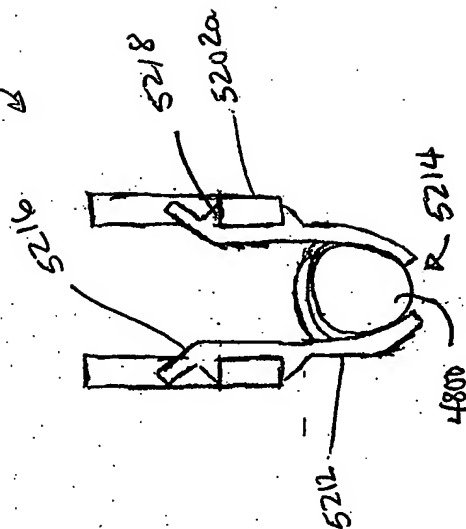
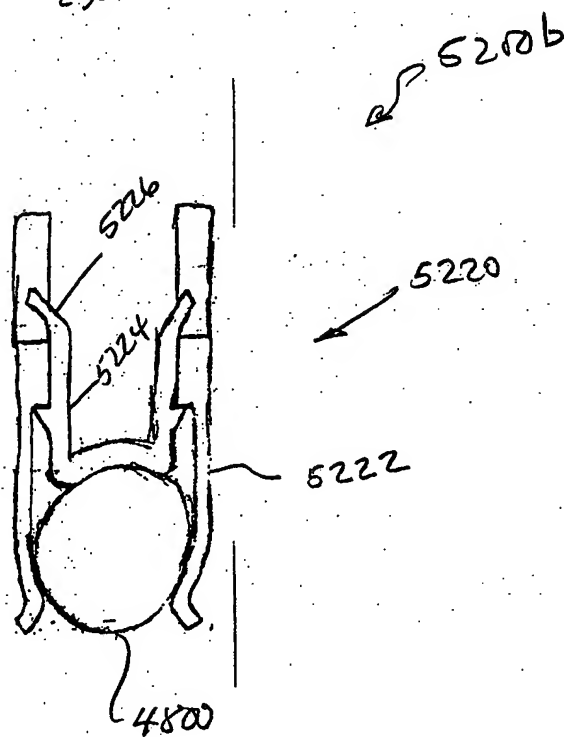
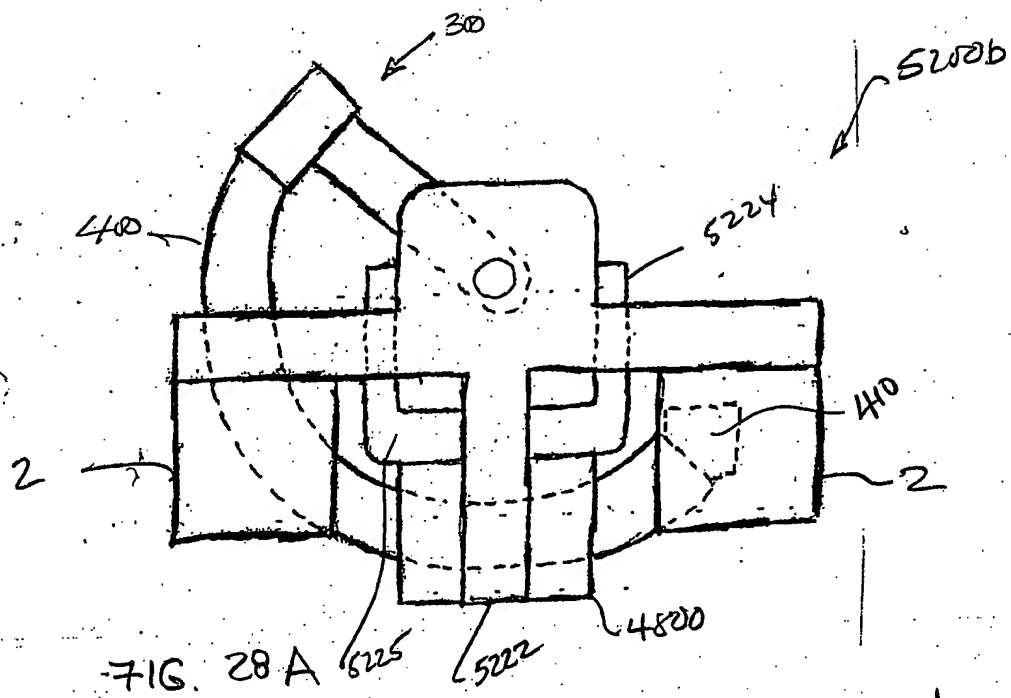
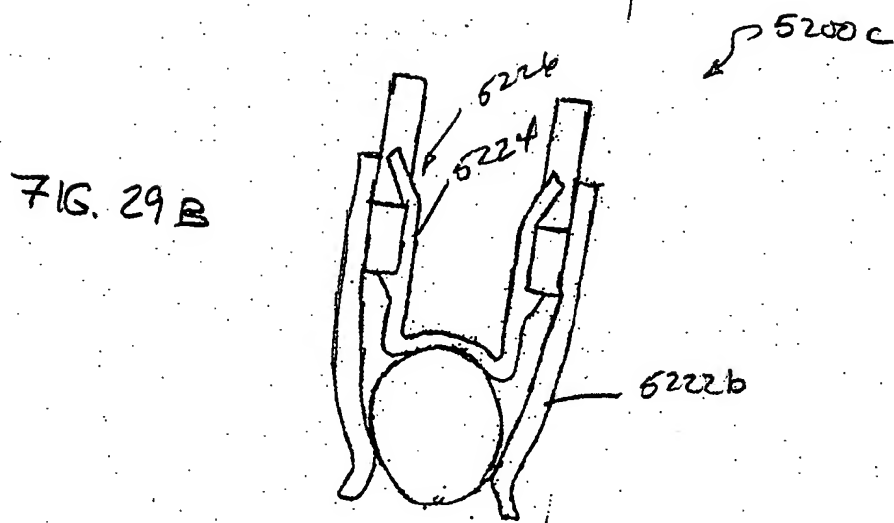
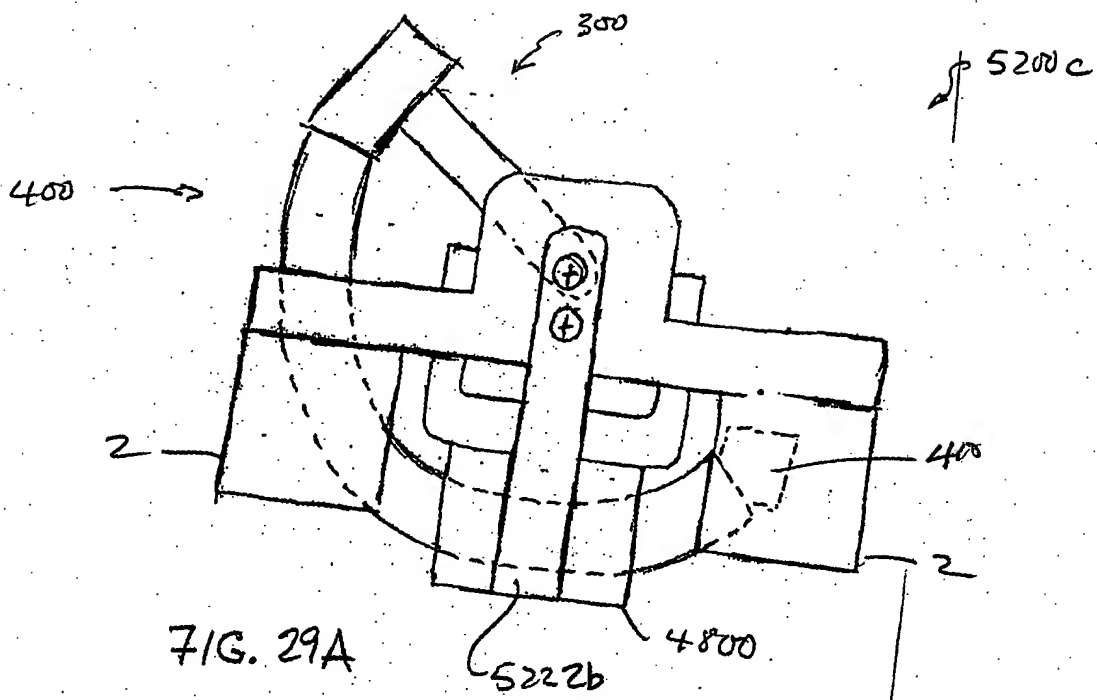


FIG. 27C





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